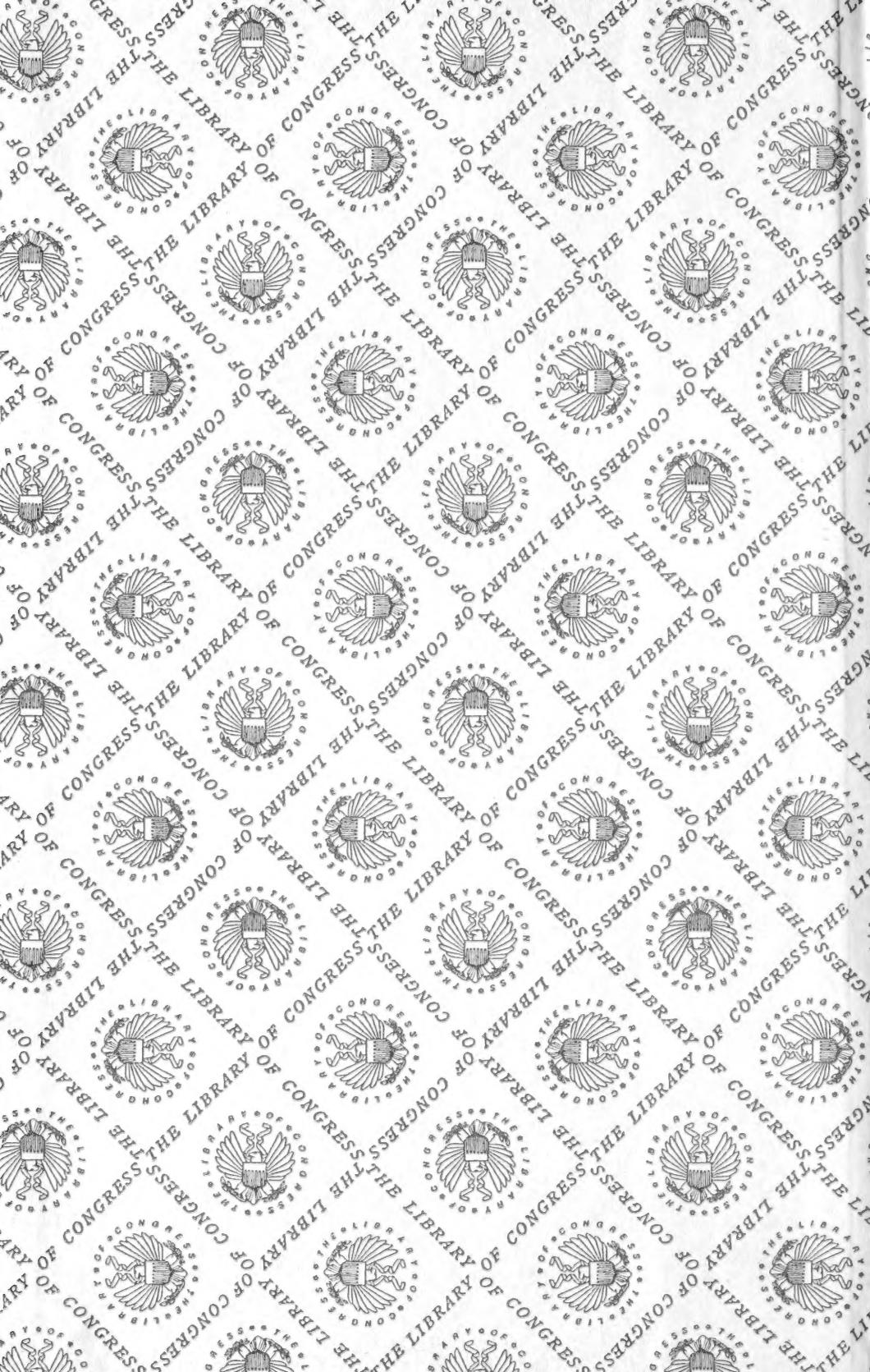
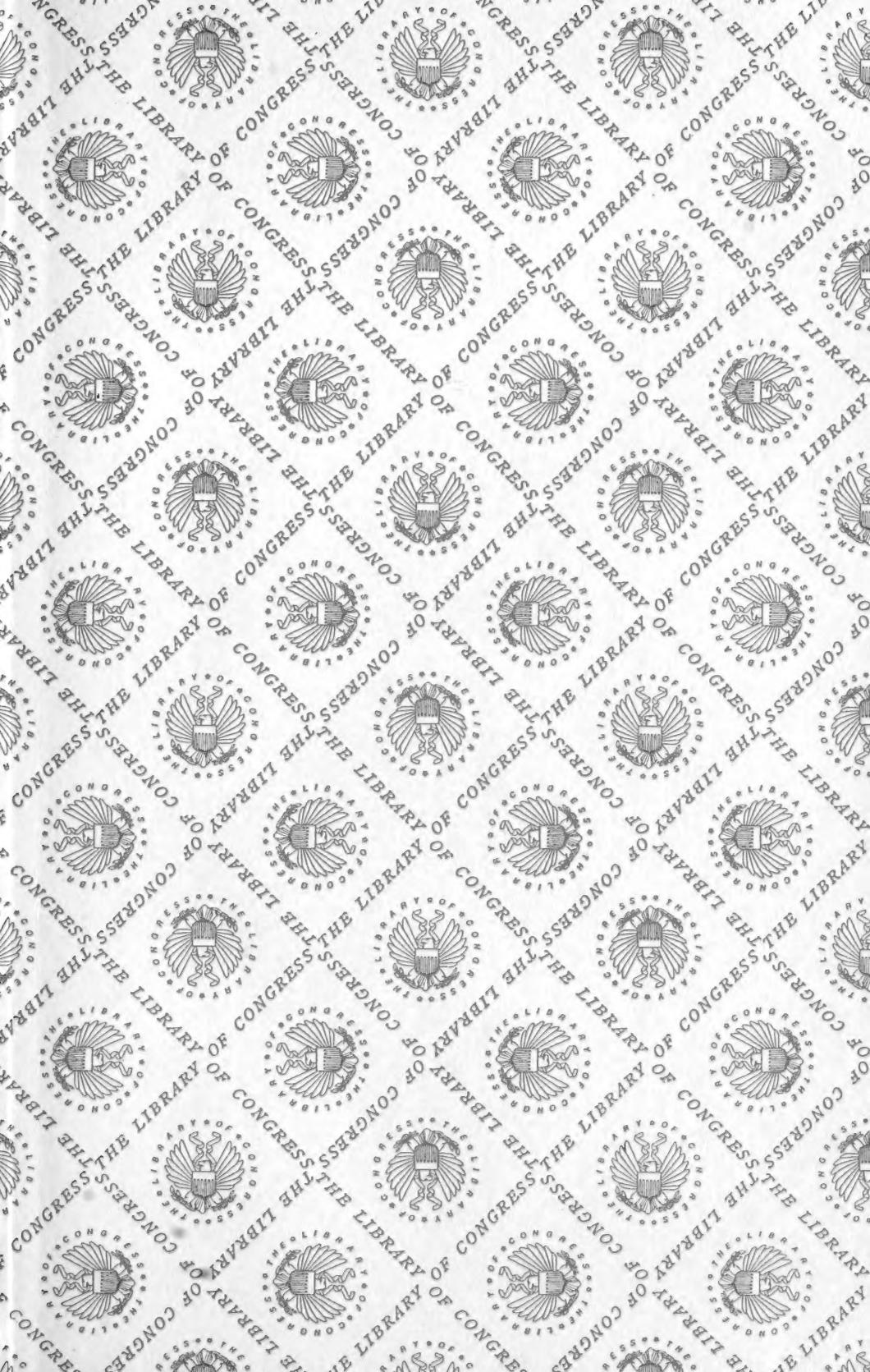


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BULLETIN No. 8

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF ANIMAL INDUSTRY.

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INVESTIGATIONS

CONCERNING

INFECTIOUS DISEASES AMONG POULTRY.

BY

THEOBALD SMITH, Ph. B., M. D., and VERANUS A. MOORE, B. S., M. D.,

UNDER THE DIRECTION OF

Dr. D. E. SALMON,
CHIEF OF THE BUREAU OF ANIMAL INDUSTRY.



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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., June 29, 1895.

SIR: I have the honor to transmit herewith a report of Drs. Theobald Smith and Veranus A. Moore concerning investigations of certain infectious diseases of poultry. This subject is one of great interest and importance to most people of this country who are engaged in agricultural occupations, and particularly to all who make a specialty of some branch of the poultry industry. There are a vast number of our citizens engaged in the production of poultry and eggs, either for their own use or for sale, and most of these at one time or another suffer losses and have their operations interfered with by outbreaks of infectious diseases. Such diseases are as destructive with birds as with other kinds of animals, and until recently they have received little systematic study from competent scientific men.

The investigations, of which an account is presented in this bulletin, open up a new field for scientific inquiry. While the work which has been accomplished is but the first step, it reveals how little has been known and how much is to be learned in regard to the diseases of birds. The greater part of the losses from infectious diseases in the poultry yard may probably be prevented by the intelligent application of proper sanitary measures. Until the nature of these diseases is clearly understood, however, it is impossible to intelligently formulate preventive measures or to prescribe successful methods of treatment.

The financial investment in domesticated birds is of such magnitude, and the products are marketed in such vast quantities, and are so essential to the well being of every citizen that the problems affecting this industry are deserving of the most careful study and investigation. The researches herein reported have been made in the hope and expectation that they would be continued until practical means of preventing the common infectious diseases are placed within the reach of all engaged in the poultry industry.

Very respectfully,

D. E. SALMON,

Chief of Bureau of Animal Industry.

Hon. J. STERLING MORTON,
Secretary of Agriculture,

LETTER OF SUBMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., April 2, 1895.

SIR: I have the honor to submit herewith some preliminary studies of infectious diseases among poultry, including fowls, turkeys, and pigeons. Though these studies have not yet reached that state which permits us to recommend any well-defined preventive or curative measures, it is to be hoped that they will give those immediately interested a better insight into the nature of some of these diseases, besides furnishing investigators throughout our country a more secure basis for further experimentation.

Very respectfully,

THEOBALD SMITH,
Chief of Division of Pathology.

Dr. D. E. SALMON,
Chief of Bureau of Animal Industry.

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INVESTIGATIONS CONCERNING INFECTIOUS DISEASES AMONG POULTRY.

AN INFECTIOUS DISEASE AMONG TURKEYS CAUSED BY PROTOZOA (INFECTIOUS ENTERO-HEPATITIS).

By THEOBALD SMITH.

[Plates I to V, inclusive.]

In the fall of 1893 the writer received from Mr. Cushman, of the Rhode Island Experiment Station, the organs of an adult turkey in which the liver and the cæca were very extensively destroyed by a peculiar disease. A second case was sent by Mr. Cushman in April of 1894, in which the same disease was recognized. In June of 1894 the writer found the same affection in a very young turkey in western Massachusetts. The changes induced by this affection were so peculiar and yet so uniform in these three cases that a specific infectious disease could not but suggest itself on even superficial examination. A careful study of this material convinced the writer that the cause of this disease was a protozoan parasite not hitherto recognized. It was, therefore, deemed best to investigate the disease more thoroughly, and for this purpose the writer spent three weeks at the Rhode Island Station during the month of August. The turkeys were obtained by voluntary contribution and by purchase, mostly from localities within 5 to 10 miles of Kingston, R. I. Some came from a greater distance. In this work I was greatly assisted by Mr. Cushman, in charge of the poultry work, and by Professor Flagg, director of the station. The chemical apparatus of the station was placed at my disposal by Professor Wheeler.

With the aid of the apparatus brought from Washington and that on hand I was enabled to make a careful examination of about 50 turkeys. Of these, 18 were affected with the specific protozoan disease, and many of the rest were affected with other diseases, briefly referred to under each case.

The short space of time which could be devoted to this subject did not enable the writer to do more than to make a preliminary study of the disease and its cause and to collect material for further study.

Now that a firm basis for the investigation of the practical problems has been secured, it is to be hoped that the investigation will be prosecuted during the coming summer, both at the experiment station of the Bureau and elsewhere. The publication of the work thus far done will also enable the various experiment stations to make observations on the communicability of the disease and on means for its eradication.

The external appearances of the turkeys affected with the disease do not seem to be constant, and this is not to be wondered at in view of the varying intensity of the changes found in the internal organs. In Rhode Island the disease is known as "blackhead," owing to certain peculiar discolorations which take place at the height of the disease. While it might be well to retain this as a popular name, we do not believe that all cases of blackhead have the specific disease herein described, nor do all turkeys affected with this disease manifest the appearances of blackhead. More extended study is needed before we may rely upon symptoms as a means of recognizing this disease during life, especially as there are several other affections of turkeys quite distinct from this concerning which there exists as yet very little definite information.

As brought out more fully further on, the disease may be recognized by anyone who has made himself familiar with the salient facts in this article by an examination of the organs of the diseased turkey after death.

Among the symptoms which may be expected to appear sooner or later, diarrhea probably occupies a prominent place. The disease of the caeca is responsible for this. Yet we may have diarrhea with at least one other disease of the caeca, and the presence of tapeworms may also cause this disturbance. Emaciation was not constantly present in the cases dissected by me. As it may accompany other wasting diseases, it can not be depended upon as a sign of this affection.

Leaving this part of the subject we shall turn to those characters of which the investigations have given some definite information.

SPECIAL CHARACTERS OF THE DISEASE.

The disease seems to attack turkeys when quite young. In early June I found a turkey about 3 weeks old (No. 3) in which the disease had already made considerable progress. It seems, moreover, as if the disease were contracted only by the young, because in the examination of turkeys of different ages the oldest showed lesions of the longest standing; that is, such as had undergone the most extensive transformation. In general it may be said that the age of the turkey corresponded with the age of the disease process. The most serious and extensive destruction of tissue was noticed in the turkeys in fall. In midsummer the disease was making most progress; that is, it was freshest and the microparasites present in great numbers. It is probable that the delicate tissues of the young are best adapted for

the temporary habitat and rapid multiplication of this parasite. In this regard it simply follows the rule observed by large numbers of parasites, whose most destructive action is visited upon the young.

The primary seat of the disease are the cæca. From these the liver is invaded. Other organs are not attacked.

The cæca of the turkey in the normal condition are represented by two blind tubes opening into the intestine at a point about 6 inches from the cloaca (Pl. II, fig. 1; Pl. I, fig. 1). They are directed toward the head, the blind end being nearer the gizzard than the opening into the intestine. They lie on either side of the latter, closely bound to it by mesenteries. The tips only are free. The length of the cæca varies according to age. In turkeys 3 months old they are about 8 inches long, while in those nearly a year old they are from 10 to 12 inches long.¹

The cæca are of nearly the same diameter as the intestine. When only slightly filled they are 4 to 5 mm. (one-sixth inch) in diameter. They may, however, be distended with feces to three times this diameter. The structure of the walls seems to be identical with that of the intestine to which they are attached. The communication between cæca and intestine is somewhat contracted. Near it in each tube there is a convex elevation about one-half of an inch long which is composed of lymphatic tissue. Its surface is usually deeply pigmented. Other lymphoid structures are represented by small elevations only three-sixteenths of an inch in diameter, usually pigmented and scattered sparsely over the mucous membrane. The mucosa itself is folded in longitudinal ridges, which are frequently pigmented.

The cæca usually contain feces in greater or less abundance which are pasty, homogeneous in texture, and greenish in color. Under the microscope they appear to be composed mainly of bacteria. When the feces have a softer consistency numerous flagellates of several distinct forms may be detected in active motion in the feces of the recently killed bird. They appear to increase in number with the increase in the watery constituents of the feces.

Coccidia have also been detected. They were present in No. 3 and in another young turkey of the same flock which died from some unknown cause. In the cæcal contents of No. 17, which was free from disease, similar bodies were found, the largest measuring 32 by 18 μ . They were in form and structure like *Coccidium oviforme* of the rabbit's liver, and perhaps identical with the form seen in the cæca of fowls by Rivolta and Silvestrini and named *C. tenellum* by Railliet and Lucet.² It is very improbable that these bodies stand in any genetic relation to the true microparasites of the disease herein described.

The lesions of the cæca are in substance a thickening of the wall,

¹ The length of the intestine from gizzard to opening into cæca is about 5 feet for turkeys 3 months old and 8 feet for those nearly a year old.

² Zoologie Medicale, second edition.

followed in most cases by a destruction of the epithelium and deeper portions of the mucous membrane. This destruction results in the outpouring of a coagulable fluid into the tube (Pl. I, fig. 1; Pl. II, figs. 2, 3, and 4). The thickening of the wall may vary considerably in extent from case to case. It may be uniform over the greater portion of the tube or it may be limited to circumscribed patches. The commonest seat of these lesions is near the blind end of the tube, where it evidently starts and from whence it spreads to other portions. Not infrequently only one caecum was found diseased, the other normal. This exemption was not due to mildness of the attack, for the other caecum may, in such cases, be the seat of very extensive changes. Thus of 18 cases only 11 had both caeca involved.

The affection of the caecum is due primarily to the multiplication of the microorganism to be described below, within the walls of the caecum. The multiplication may take place chiefly in the mucous membrane, or in the submucous tissue, or it may, though rarely, extend into the muscular coat. The thickening of the wall is the result of several processes—the multiplication of the parasites, the increase of the normal tissue elements, and later on the accumulation of masses of small cells and some giant cells.

In the early stages of the invasion the adenoid tissue between the tubules and in the submucosa becomes greatly increased, owing to the presence of large numbers of microparasites of round or slightly oval outline and from 6 to 10 μ in diameter which stimulate the proliferation of the tissue cells. Numerous mitoses have been seen in this stage. The parasites seem to occupy the meshes of the adenoid tissue either singly or in groups or nests (Pl. IV, figs. 1, 2). In these meshes they are soon enveloped in cells acting as phagocytes, so that the appearance of an intracellular habitat of the parasites is suggested, which is, however, only an appearance and not demonstrated by further study.

The presence of the parasites in this reticulum probably stimulates also the accumulation of lymph cells within the spaces, in virtue of which the mucous membrane is thickened. In this early stage of invasion the epithelium, both of the tubules and of the surface, remains unaffected. The parasites do not invade the epithelium at any time.

As the disease progresses there is a continued increase in the cellular elements of the mucous and submucous coats and a gradual invasion of the muscular coats. Here the bundles of fibers of the circular coat are thrust apart by masses of cells, so that this coat also becomes greatly thickened. The inflammation finally extends to the serous covering, where the blood vessels become greatly dilated and give the caecum a congested appearance. In cases of ordinary severity the wall of the caecum, not more than 0.2 to 0.5 mm. (one one hundred and twenty-fifth to one-fiftieth inch) thick normally, according to distention, becomes 2 to 3 mm. (one-twelfth to one-eighth inch) thick.

With the progress of the disease the mucous membrane may be shed and a coagulable fluid poured out into the cæcum. In some cases it appears in isolated masses, which adhere to certain spots of the mucous membrane. In others, this exudate fills the entire tube with a yellowish-white mold (Pl. II, figs. 2, 3). This is built up in concentric layers, and consists of a mixture of blood corpuscles, fibrin, and masses of small round cells, in variable proportion.

In the further progress of the local disease it is not improbable that bacteria are also concerned. The exudate contains immense numbers of them and the denuded mucosa furnishes a favorable place of entry. It is otherwise difficult to explain the continued increase in thickness of the walls of the cæcum after the mucous membrane has been shed. This continued increase in thickness is due to an extensive infiltration of small round cells and the presence of some giant cells. Parasites in this advanced stage are scarce, and usually recognizable only as vacuole-like bodies within the giant cells.

The thickening of the wall is associated in some cases with an extension of the inflammation to the contiguous wall of the intestine, which is thereby firmly attached to the cæcum. Yellowish exudates are sometimes found outside of the diseased cæcum on its serous covering and they bind it inextricably to the other cæcum or to the intestine or attach it to the abdominal wall. In these stages the microparasite is not found. It seems to have done its work by destroying the mucous membrane and to have left the field for miscellaneous bacteria.

In no case were other regions of the digestive tract affected. Though the inflammation set up in the affected cæcum may have extended through the wall to the contiguous intestine and caused the formation of a large amount of neoplastic tissue between the adjoining tubes, the disease did not invade the submucous or mucous membrane and the specific parasites were absent.

Associated with this peculiar disease of the cæca is a more or less serious disease of the liver. In the 18 cases of protozoan disease the liver was very extensively diseased in 16. In two cases only small sclerotic foci were present and in these the lesions of the cæca were evidently at a standstill, or in a condition of active cicatrization. The affection of the liver is in fact a valuable means of recognizing this disease, for wherever it is present we may safely infer that we have the protozoan disease before us. The reverse is, of course, not necessarily true. The disease of the cæca may be so restricted that the secondary liver disease does not appear. Nevertheless, the value of the liver affection is indicated by the fact that in the few cases of cæcal affection in which the disease was of a different character no changes in the liver were manifest (Nos. 25, 41).

The appearance of the liver in the average case of this disease is very striking. The organ itself is enlarged in proportion to the amount of tissue involved. Twice the normal size is probably near the truth,

although but one was weighed to accurately determine this point. In this case (No. 52) the liver weighed 10.7 ounces.

Over the surface of the organ are distributed roundish, discolored spots, distinctly demarcated from the surrounding tissue. These spots vary much in numbers, in size, and in appearance. They may be distributed uniformly over the whole surface of the liver or they may be limited in number to a few. They vary from 3 to 15 mm. (one-eighth to two-thirds inch) in diameter. Inasmuch as a detailed description of the different cases examined is given in the appendix, to which the reader is referred, only a general description will be attempted here.

In endeavoring to group these spots according to their general appearance we find several types, which, as will be seen below, correspond to different conditions of the diseased tissue. We have in the early most active disease process, spots like those photographed and figured in Pls. I and II. They are sharply defined circular areas of a lemon yellow or an ocher yellow color. A careful scrutiny shows that the spot is not homogeneous in structure, but made up of a delicate network of yellow, as shown in the plates. This yellow substance represents dead tissue.

In another class of spots we have in place of the yellow meshwork an entirely different appearance. The spot is of a mottled brownish color and contrasts only slightly from the surrounding liver tissue by its darker color. It may contain a central yellow nucleus of dead tissue and a narrow outer border of the same character, or the border may be a dark brownish circular line. The entire spot has an indistinct appearance and is flattened or even slightly depressed below the surface. In some cases the spots assume still another aspect. They are uniformly whitish and shade off somewhat gradually into the surrounding tissue.

If sections be made of the affected organ it will be noticed that these surface spots represent masses of liver tissue in the same condition. The spots are simply the places where these disease foci intersect the surface. Some are found deeply embedded in the liver tissue, and therefore not visible on the surface. The lesion of the liver is thus represented by few or many foci of disease having in general a spherical form and appearing on the surface of the organ as round spots (Pl. III, fig. 1). Occasionally the lesions become more extensive and the death of large portions of liver tissue follows. This is well shown in fig. 2 of Pl. III, where a large volume of liver tissue is transformed into a yellow cheesy substance.

The changes in the liver are most easily explained by assuming that the microparasites are conveyed by the blood directly from the diseased cæca into the liver and there deposited in different places, where they begin to multiply and spread in all directions, thereby forming the spherical foci of disease which appear as circles on the surface of the liver. This theory is borne out by the results of the microscopic examination.

In sections of hardened tissue from the liver in which the disease has but recently begun the affected regions are invaded by large numbers of protozoa, which occupy a kind of reticulum formed probably from the connective tissue stroma. The liver cells have partially or wholly disappeared from these foci. The parasites occupy the meshes of the tissue either singly or in groups. The reticulum is provided with a small number of nuclei, some of which are closely applied to and curved partly around the parasite. The blood vessels are usually much dilated and filled with red corpuscles.

The yellow masses observed with the naked eye in the surface spots in many cases are shown to be patches of an amorphous substance which takes nuclear stains very feebly, the aniline colors not at all. It may be described as a coarse network in the meshes of which small cells, and very rarely parasites, are seen. This substance I assume to be the result of coagulation necrosis of the liver cells by which they have lost their nuclei and have become fused into a formless mass. I at first was inclined to look upon it as fibrinous in character, but the discovery of transitional stages confirms the view that it is made up of dead liver cells. It is probable that the plugging of blood vessels in the liver by parasites carried from the cæcum is the cause of the necrosis, since such plugs or thrombi are not uncommon in sections of the diseased spots.

With the appearance of the microparasites reactive changes begin at once which complicate the process. We have at the outset an active multiplication of the microparasites which take the place of the original liver tissue, and a process of coagulation necrosis going on at the same time. Soon multinucleated (or giant) cells appear which not only take in the microparasites but which are also engaged in removing dead tissue. At least their collection in groups around and within the necrotic areas lead us to assume this. Not infrequently they are grouped around what appears to be a plugged vessel, or else they occupy the lumen of the vessel itself. The presence of round or oval vacuoles within these large cells indicates that parasites have been taken up (Pl. V, fig. 3).

In still older cases the diseased spots are found more or less filled with small round cells which may have passed into the dead regions from the blood vessels. In all cases the latter are more or less enlarged, and they seem to encroach upon the liver tissue, thus filling in part the void produced by the cell death and giving the surface of the liver a brownish mottled appearance wherever the disease spots are.

The processes of advancing disease and necrosis or death of tissue on the one hand, of repair on the other, seem to go on side by side, now one now the other predominating. Of the process of repair case 16 is a very good illustration. In this bird the liver tissue was undergoing sclerosis associated with the true healing process, which is referred to more in detail in the appendix.

Besides the lesions directly due to the microparasites in the caeca and in the liver, there were no changes observed to indicate that the parasite was multiplying in other parts of the body. Even where the disease was extending locally from caecum to adjoining intestine, the process was not due to any advancee or invasion of the parasites themselves.

Regularly recurring changes in other organs due indirectly to the destruction of liver tissue, and to the loss of the caeca as important aids in the process of absorption were not manifest.

The blood of many of the cases was examined before the bird was killed in order to detect any existing blood infection. No disease of the blood was observed, however, excepting in No. 16, in which a pronounced leucocytosis was present. In this case the disease was disappearing and the liver undergoing sclerosis, as stated above. In a small number of cases, some diseased, some healthy (Nos. 5, 6, 12, 15, and 33) flagellates were detected in variable numbers. They were evidently not related in any way to the protozoan disease. These bodies (Pl. V, figs. 6 and 7) are spindle-shaped and made up of an oval, feebly granular, central body (nucleus) around which the cell body forms two lateral wings and two tapering extremities finely granular in structure. Motion was not detected at the temperature of the surrounding air. Higher temperatures were not applied. The nucleus of this flagellate is about 25μ long and 6μ broad. The entire organism is 65 to 70μ long. When the blood is dried in thin films upon cover-glasses and stained, these bodies appear in many instances much broader than in the fresh condition. This would suggest that the organism may be a flattish body rolled up so as to bring the two lateral margins near together.

The results of the examination of turkeys made thus far indicate that the disease may follow several courses:

(1) After a certain period of disease regenerative processes begin which tend toward a permanent recovery.

(2) The disease may proceed so rapidly from the very start that the affected turkeys die early in life.

(3) The disease may come to a standstill, but the amount of dead tissue in the caeca and liver may be so great as to favor the entrance of bacteria which are responsible directly for the death of the bird late in the summer or fall.

The direct action of the microparasites upon the body seems to lead to a mechanical destruction of tissue. In this respect they differ from most bacteria, which may combine a locally destructive process with a general poisoning of the body. The extensive destruction of liver tissue combined with the loss of the use of one or both caeca in the digestive process must of necessity lead eventually to weakness and emaciation. The feebler resistance of the affected animals was brought to our notice last summer by their speedy death after a long ride or a few days of confinement.

As regards the spontaneous cure of infected birds we may regard it as probable that only those in which the disease comes to a standstill before it has made much headway are likely to recover. In those in which much destruction of tissue has taken place complete recovery is impossible. The dead masses embedded in the caeca or the liver, or both, form a nidus for bacteria of certain kinds. These penetrate into the pleuroperitoneal cavity and there set up inflammation and finally lead to a general septic infection of the body. The immediate effect of the disease may thus be very slight at the outset and the symptoms develop slowly. The great increase in the size of the liver indicates a certain amount of compensation for those portions which have been destroyed.

The effect of the disease on the flesh of turkeys killed in the early stages is not recognizable. In a few cases which died from a severe form of the disease the flesh had a mahogany color, probably due to retained biliary substances.

THE MICROORGANISM.

(*Amœba meleagridis n. sp. 1895.*)

In the 18 cases of this disease which came under observation either the liver or the caeca, or both organs, were examined microscopically in 14. In these organs certain microorganisms were detected in all but one case and associated directly with the peculiar disease process already described. In those cases in which the disease was recent, or at its height, the parasites were very numerous in the affected tissues, while in those in which the disease process was far advanced, and associated with degenerative or regenerative changes, the parasites were found with difficulty. These facts are summarized in the following table:

Table showing the post-mortem results (microorganism) in 18 affected turkeys.

No. of turkey.	Approximate age.	Source.	Manner of death.	Extent of cecal disease.	Microparasites in—		Stage of disease.
					Extant of liver disease.	Cecum.	
1	6 months	Rhode Island...	Died.....	Extensive, both ceca.....	Extensive	Scarce	Old.
2	10 months	do.....	do.....	Extensive, one caecum.....	do.....	do	Do.
3	3 to 4 weeks	Massachusetts...	do.....	Extensive, both ceca.....	Moderate	Abundant	Recent.
4	3 months	Rhode Island...	do.....	do.....	Extensive	Not examined	
5	2 months	Farm A, R. I....	Killed.....	do.....	Moderate	do.....	
6	2 to 3 months	Farm B.....	Died.....	Extensive, one caecum.....	Slight	do.....	Moderate
8	do.....	Farm C.....	Died (confined).....	Moderate, both ceca.....	Very slight	None seen	Do.
9	3 months	Farm D.....	Died.....	do.....	Extensive	Scarce	Probably healing.
14	do.....	Farm G.....	do.....	Extensive, both ceca.....	do.....	Moderate	Advanced.
16	do.....	do.....	Killed.....	do.....	Sclerosis.....	Scarce	Do.
19	3 to 4 months	Farm J.....	Injured in catching.....	Moderate, one caecum.....	Extensive	do.....	Healing.
23	do.....	Farm L.....	Died.....	Extensive, one caecum.....	Very slight	do.....	Advanced.
26	3 months	Farm G.....	Killed.....	do.....	Extensive	do.....	Advanced.
34	2 to 3 months	Farm K.....	Died.....	Moderate, one caecum.....	do.....	do.....	Active.
35	do.....	do.....	do.....	Moderate, both caca.....	do.....	do.....	Do.
36	3 months	Farm N.....	do.....	do.....	do.....	do.....	Do.
51	5 to 6 months	Farm B.....	do ¹	Extensive, one caecum.....	Moderate	do.....	Old.
52	do.....	do.....	do ²	Slight, one caecum.....	Extensive	do.....	Do.

¹ Death probably from septic infection.
² Death probably from injury during journey.

In the examination of the parasite within the tissues minute particles of the latter were crushed in iodized amniotic fluid or in normal salt solution. Sections were also made. Unfortunately, a freezing microtome was not at hand, nor any warm stage for determining the effect of higher temperatures on these bodies.

The most frequent appearance presented by the parasites under these circumstances was that of round homogeneous bodies with a sharply defined, single contoured outline (Pl. V, fig. 1). Their appearance suggests that of the myelin bodies found in the cells of the pulmonary alveoli, not refrangent enough to represent oil globules, but too homogeneous to represent the ordinary protoplasm. Within these bodies and situated somewhat eccentrically is a group of very minute granules, probably representing a nuclear structure. These forms were encountered in those cases in which the parasites were found most abundant in sections of hardened material (Nos. 5, 24, 26); also in one case in which repair was evidently going on (No. 16). They vary somewhat in size. In Nos. 5 and 16 those that were measured were 8 to 10 μ in diameter. In No. 26 they were 12 to 14 μ in diameter. In No. 34 some of the bodies were oval, having diameters of 12 and 15 μ , respectively. They are thus distinctly larger than the parasites within the tissues, which have undergone the hardening process. The latter are from 6 to 10 μ in diameter. This difference may be due to shrinkage, on the one hand, and on the other to a slight flattening of the bodies by pressure in the fresh preparations. These peculiar homogeneous bodies were found, as a rule, free in the crushed preparations, although occasionally giant cells were detected which contained a number of them. The cell nuclei of the giant cell were not visible in the fresh condition. Numerous coarse granules were embedded in its protoplasm, less frequently fat globules.

Besides the homogeneous bodies, organisms of nearly the same size but with uniformly granular protoplasm were also found, chiefly within giant cells. These were not infrequently broken and rims or fragments of protoplasm were left attached to the parasite (Pl. V, fig. 2).

For the study of hardened material the tissues were removed in most cases immediately after the bird had been killed, and placed in one or more of the following fixing and hardening agents: 95 per cent alcohol, saturated solution of corrosive sublimate, Foa's solution (equal volumes of the preceding solution and a 5 per cent solution of bichromate of potash), Flemming's solution (undiluted). The tissues were fixed in the three last mentioned during one day, then washed in running water during another day and treated with ascending strengths of alcohol, according to the usually prescribed rules. The tissues were then passed through absolute alcohol and chloroform, infiltrated in paraffin, and the sections cut dry. Various staining reagents were employed, including Delafield's hematoxylin and eosin, alum carmine, Biondi's triple stain, saffranin, Gram and Gram-Weigert's stain. Of the fixing and hard-

ening agents I am unable to consider any one as superior to the other in bringing out the parasites in the tissues when subsequently stained. By this I do not mean to infer that there was no preference when their action upon the tissues is considered, for here they manifested their respective peculiarities already well known. But the fact remains that now the one, now the other, tissue demonstrated the parasites best. In general, hardening in corrosive sublimate and alcohol and staining in Delafield's hematoxylin and in eosin proved the most successful. The bodies were, however, brought out with other dyes, such as methylene-blue, with variable distinctness.

The microparasites as found in sections of hardened tissue are spherical or slightly oval bodies from 6 to 10 μ in diameter. In sections stained with hematoxylin and eosin they have a homogeneous bluish-red tint, feebler than that of the tissue nuclei of the host. They are, however, distinctly seen, and the advantage of a more intense stain is questionable. In most of these bodies a minute, distinctly blue ring is seen situated centrally or somewhat eccentrically and representing the nucleus. This is about 2 μ in diameter. With high powers a very minute (nucleolar?) point may be seen in some parasites within the nucleus. Other differentiations of structure were not noticed.

The presence within the connective tissue spaces of parasites in groups of two or more individuals, as well as of single individuals, makes it highly probable that at least in the early stages of the disease there is active multiplication of these bodies. This appears to go on as a simple process of division, if we are to judge from the grouping within the tissues. The variation in the number of individuals composing such groups, as well as the absence of any common membrane inclosing each group, indicate at least that the multiplication is not an endogenous segmentation such as belongs to the sporozoa.

This brings us to a consideration of the nature of these protozoa. Their simple structure would lead us to class them with the amoebæ, of which one form is now known to produce a similar disease in the human subject (amoebic dysentery). Amoebæ are also not uncommon inhabitants of the large intestine of man and certain animals. The peculiar homogeneous structure and rigid outline of the organisms before us as they appear when examined in tissues directly from recently killed animals may represent a kind of encystment in the presence of the adverse forces of the animal tissues. In the sharply defined vacuoles within the giant cells this rigid form seems to have left a permanent impression (Pl. V, fig. 3).

The relation which the parasite bears to the microparasites found in external tumors or warts (*Molluscum contagiosum*) of fowls, or to those flagellates which are associated with diphtheria in fowls, or finally to the diphtheria referred to in text-books as due to gregarinosis may be a very distant one. Yet, in the present state of our information concerning these parasites, it is worth while to be on our guard before

making positive assertions, and leave it to future work to decide if any relationship exists.¹

We are naturally led, in a consideration of this microorganism, to inquire what relation it bears to the tissue cells of the host. Is it an intracellular parasite during any portion of its parasitic existence? The evidence brought forward here would lead me to state that it lives in the interstices and lymph spaces of the tissue, but not within cells. This seems certainly true of the caecum. In the liver the liver cells seem to become necrotic or else disappear so rapidly that it is impossible to determine just where the parasites begin to multiply. They do not live within the blood vessels, as they are not found within them excepting perhaps in a thrombosed vessel. They must, therefore, occupy the place of the liver cells. It is probable that they begin to multiply in the connective tissue adjoining the blood vessel, and simply crowd out the liver cells, leaving the connective tissue stroma of the lobules in whose meshes they are found.

Their presence within giant cells is seen in almost every infected organ subject to examination. In teased preparations of the fresh tissues they are frequently found with remnants of the inclosing cells still attached. This intracellular condition is, however, a purely passive one so far as the parasite is concerned.

The fate of the microparasite within the tissues of the host seems to tend toward destruction. Both the death of the tissue itself and the repair seem to lead to the disappearance of the parasites. In most cases there may be seen in the same section a partial dissolution of some of the bodies, while others are still in good preservation. Evidently their life within the tissues is not very long. The attack upon them by large giant cells in the later stages of the disease has already been mentioned.

A discharge of the microparasites which escape destruction probably takes place from the walls of the caecum, when these break down, into the contents, in which they are carried outward. A similar discharge may take place from the liver through the bile ducts into the intestine. Another way of dissemination would be in the death of the diseased turkey and the dissolution of its body whereby the organisms are set free. Speculations in this direction are, however, of little value until experiment shall have determined whether turkeys are infected directly from sick turkeys, from healthy, immune or recovered turkeys, in whose caeca the parasite may perhaps vegetate indefinitely, or from some external source.

The close analogy existing between this disease and that of amœbic dysentery in the human being deserves at least a brief statement in these pages. The occurrence of amœbae in intestinal affections of man was noticed as far back as 1875 by Lösch. Since that date it has been

¹ The presence of numerous flagellates in the caeca of healthy turkeys has already been pointed out on page 9.

the subject of many investigations.¹ Two of the more exhaustive investigations which have appeared are those of Councilman and Lafleur² and of Kruse and Pasquale.³

The points of analogy between the avian and the human disease are that in both there is an affection of the intestine (large intestine in man, cæca in turkeys) associated with liver disease due to amœbæ.

The intestinal wall in amœbic dysentery is greatly thickened, owing to an oedematous condition. It is also thickened in circumscribed areas and contains cavities filled with gelatinous-looking pus. The amœbæ vary much in size and contain vacuoles. They are found in variable numbers in the bottom of the ulcers and in the discharges. The large numbers of amœbæ found in the intestinal contents led Councilman and Lafleur to infer an active multiplication therein. The presence of the parasites within the submucosa is described by these authors in one case⁴ only.

In the turkey we have seen that the parasites are always present in the connective tissue spaces of the mucous and submucous membrane. Their presence in the contents of the cæcum is highly probable from examinations made last summer but not definitely settled, as it is difficult to recognize the parasites in the feces.

The microparasite of the turkey disease differs from the *Amœba dysenteriae* in being quite uniform in its appearance, varying but slightly in size (from 6 to 10 μ in diameter) and in being free from vacuoles. Movements characterized as amœboid have not yet been demonstrated.

The liver affection in man appears usually as an abscess. In turkeys it appears as a variable number of foci in which the microparasites may be present in great abundance. The difference in the nature of the lesions must be largely attributed to the different reaction of the tissues of birds toward injuries. This brief statement must suffice to point out the analogy between the human and the avian disease, and the tracing of any further relationships must be left to those specially interested in this subject.

Before concluding the discussion of the microparasite of this disease I desire to refer briefly to peculiar organisms found in the lumen of the tubules of the cæca. Fig. 5 of Pl. V depicts these bodies very well. The figure represents a cross section of one of the tubules from the diseased cæcum of No. 26. It will be noticed that the tube is packed with minute organisms of a roundish or oval outline provided with a more deeply stained body, probably a nucleus. The organisms, after

¹For a good résumé, with citations of the literature, the interested reader is referred to Schuberg: Centralblatt für Bakteriologie, XIII (1893), Nos. 18 to 22 inclusive.

²W. T. Councilman and H. A. Lafleur. Amœbic Dysentery. The Johns Hopkins Hospital Reports, II (1890-91), pp. 395-548.

³W. Kruse u. A. Pasquale, Untersuchungen über Dysenterie und Leberabscess, Zeitschrift f. Hygiene, XVI (1894), pp. 1-149.

⁴Loc. cit., p. 490.

being fixed in corrosive sublimate and hardened in alcohol, measure from 4 to 5 μ in diameter. The nuclear body, though it appears round or oval in the figure, is actually more complex in form. Careful focusing with a high power ($\times 1,000$) shows a spindle-shaped or oval body which may be bent like the arc of a circle. Its shortest diameter is about 1 μ . At first I was inclined to look upon these bodies as blood corpuscles which had escaped into the tubules during the disease process from the cavity of the cæcum. This view is made very improbable by a number of facts. The red corpuscles in the capillaries of the same section are quite different from these bodies, both as regards the color of the hemoglobin, and the form, size, and structural appearance of the nucleus. These problematical objects assume a bluish-pink color when stained in hematoxylin, and the nucleus stains quite deeply and solidly. Again, it would be difficult to understand why all the tubules should become packed with these bodies if they were blood corpuscles. Another supposition, that they might be some stage of the microparasite of this disease does not harmonize with the fact that they are found within the tubules of the healthy cæcum (No. 47). They may be absent in cases where the disease is most active (No. 3). On the other hand, they were very abundant in No. 26, and less so in No. 16.

The most satisfactory assumption is that these organisms represent one of the unicellular animals, probably a flagellate. It is interesting to note that flagellates were found in large numbers in the fresh contents of the cæcum of No. 47, in which these bodies were found within the tubules in sections of hardened tissue.

There is probably no genetic relation between this hypothetical organism and the true parasite of the disease under consideration. The latter is larger, its nucleus is smaller and simpler in form, and its behavior toward staining reagents is different both as regards the cell body and the nucleus. It would seem, on the whole, that we have here a microorganism which inhabits the cæcum and by preference the depths of the tubules. Its effect upon the health of the turkey can, of course, be only conjectured at present.

THE RELATION OF BACTERIA TO THE DISEASE.

From what has been stated concerning the presence of the protozoan parasite in the diseased tissues, there can be little doubt that they must be regarded as the cause of the disease. In the course of the investigations the possible bearing of bacteria upon the disease was not entirely overlooked and cultures mainly upon agar were made from the blood and the liver of a considerable percentage of the infected turkeys. Usually, bits of liver tissue were transferred to the culture tubes. Of the heart's blood, one or two loops were inoculated. The results obtained varied from case to case. As a general rule, tubes inoculated from birds that had just been killed remained free from growth, whereas the cultures of those which had died during the night

and were examined next morning were quite regularly fertile. The bacteria isolated from these cultures were examined by Dr. C. F. Dawson, assistant in the laboratory, and most of them found to be *B. coli communis*. Several of these were afterward reexamined by me and his results confirmed.

The absence of any uniformity in the bacteriological results as well as the appearance of *B. coli* in the organs of dead turkeys indicates that pathogenic bacteria were not associated with the protozoa in the cases examined. It furthermore warns us to be careful not to attribute to such bacteria as may be found the power of producing disease, as has been lately done by Lignières,¹ who found *B. coli* in the organs of a small number of dead fowls and claimed, without positive proof, that they were the cause of a septicæmia.

THE RELATION OF THIS DISEASE TO CERTAIN OTHER DISEASES OF POULTRY.

(1) *To other diseases of the caeca in turkeys.*—In the investigations made in Rhode Island three cases came under my observation which make it certain that not all diseases affecting the caeca of turkeys are due to the protozoa described.

Of one case (No. 7) only the intestines were obtained. One of the attached caeca was greatly distended by an exudate, similar to that found in some cases of the protozoan disease. At about 1 inch from the blind end the caecum abruptly swelled out to a diameter of $1\frac{1}{4}$ inch. The enlarged portion was about 2 inches long and very firm to the touch. The whole mass was placed unopened in alcohol. When it was subsequently opened the enlargement was found due to a firm exudate with a narrow canal passing through the center. The exudate readily peeled away from the wall, which was but slightly thickened.

The second case (No. 25) was similar to this. Only one of the caeca was affected. The notes on this case are as follows:

One caecum slightly distended by a rather firm cylindrical mass of feces encased in a whitish leathery exudate made up of concentric layers. The whole is not adherent to the mucosa. This membrane is dull, slightly congested. The wall is very slightly thickened. The liver is free from disease.

In the intestines are numerous tapeworms. Two cultures were made by placing bits of liver tissue in tubes of inclined agar. In both, colonies appeared. These were subsequently found to be closely related to *B. coli communis*.

In the third case (No. 41) both caeca were diseased, the liver normal.

One caecum was very much distended over a distance 3 inches long, attaining a diameter of three-fourths of an inch. From the serous aspect the wall appeared very hyperæmic. When the tube was slit open the distension was found due to a cylindrical mass of exudate, very firm, yellowish white. The exudate appears deposited in concentric layers. The lumen of the tube within this exudate was almost occluded. Odor strongly fecal. The exudate easily peeled away from the wall in a single mass. The wall was smooth and hyperæmic and but slightly thickened.

¹Septicæmie à coli bacille chez la poule. Compt. rend. Soc. Biol. 1894, p. 135.

This exudate was hardened in alcohol and transverse sections cut from it. These show that it is composed almost exclusively of a meshwork of amorphous material, probably fibrin. Cell groups were found in but one locality of the exudate.

The other cæcum is only slightly distended. It contains a mass of exudate about 1 inch long and one-third of an inch in diameter lying loose in the cavity. The mucosa is roughened and covered with a thin slough or exudate.

Sections of the wall of this cæcum show the mucous membrane replaced by a layer of young connective tissue cells in which tubules are absent excepting in circumscribed regions in which the remnants of the tubules are still recognizable in the amorphous mass adhering to the submucosa. The muscular coats are not penetrated by cell masses, but the vessels of these and the subserous membrane are greatly distended with corpuscles.

The small intestine of this turkey contained numerous small tapeworms.

Two agar cultures were made from heart's blood and one with a particle of liver tissue. In one of the blood cultures a number of similar colonies developed. The bacilli composing one of them had all the cultural characters of *B. coli communis*.

These three cases are of interest as indicating the existence of some other agency, probably bacterial in nature, which affects the cæca of turkeys. It differs from the protozoan disease in the entire absence of any liver affection and in the absence of any marked thickening of the walls of the cæca. The exudate is much more abundant and obstructive than in the protozoan disease. Further investigations are necessary to define more clearly the nature of this affection and its cause. A similar affection of the cæca was found in two turkeys by Von Ratz in Budapest.¹

He found much thick mucus in the mouth and esophagus. The mucous membrane of the small intestine was studded with hemorrhages and small, removable pseudo-membranes as large as lentils. Both cæca of one turkey contained a partly dried, cylindrical mass, and many minute worms 8 to 14 mm. long. In one cæcum of the other turkey the same dry, cylindrical mass, easily removable. The mucous membrane itself was covered with layers of pseudomembrane. The other cæcum was distended with gases and a grayish, semifluid mass. Both contained many roundworms.

The author does not hesitate to attribute the cæcal disease to the roundworms. This conclusion is, however, merely an inference based on the presence of worms and exudate. It is more than probable that the disease is bacterial and secondary to the catarrhal affection of the mouth.

(2) *To disease of the cæca in fowls.*—This is by no means uncommon. Zürn² considers it a form of diphtheria which may affect the various parts of the head, the digestive and respiratory organs. He states that the disease of the cæcum may appear as an independent affection in water fowls and turkeys. The disease is accompanied with great depression and weakness. The diarrhea which appears is at first semi-fluid and mucous, later it becomes fluid and is mixed with blood. The foul-smelling discharges may be followed by constipation, and then the

¹ Blinddarmentzündungen bei Truthühnern durch Ascariden verursacht. Deutsche Zeitschrift f. Thiermed., XX (1893), p. 74.

² Deutsche Zeitschrift f. Thiermed., X (1883), p. 189.

cæca and the rectum are found after death filled with yellow croupous masses in layers upon the frequently ulcerated mucous membrane. The disease lasts fourteen to twenty days or even two to three months. Often a relapse follows an apparent cure. He states that it frequently happens that the croupous or diphtheritic affection of the throat, nose, and eyes appears healed, when suddenly the intestinal disease breaks out and usually ends fatally.

The writer has seen two instances of disease of the cæcum in fowls. In one case one cæcum had its walls very much thickened. The same was true of the second case. In this the thickening was due to a reparative inflammation following the loss of a greater part of the mucous membrane. In sections of hardened tissue protozoa were not recognized. It is probable that in this fowl the disease was secondary to a diphtheritic affection of the head.

Siedamgrotzki¹ describes a disease of both cæca in a hen. They were very much enlarged. In one cæcum the wall was thickened by inflammation, but the mucous membrane was intact. In the other the wall was also thickened and adherent to neighboring organs. The mucosa was covered with a yellowish-brown, thin, pseudomembrane.

Disease of the cæca in poultry seems to be a widely distributed affection, both primary and secondary to other diseases, and evidently due to more than one agency. It is to be hoped that more will be known concerning these affections in the near future, and that the demonstration of one specific cause in the form of protozoa will pave the way for the determination of the others.

GENERAL CONCLUSIONS.

In the preceding pages we have shown that there prevails a disease of turkeys which attacks the young by preference, which runs no regular course, but varies from bird to bird in severity, duration, and termination. It begins in the cæca and thence invades the liver through the blood. Though restricted to these two organs, its action is severe enough to prove fatal to many affected turkeys. We have seen that the disease process is always associated with a protozoan parasite of very minute size, whose destructive action is due in the main to its powers of rapid multiplication within the tissues. It is probably discharged with the droppings of the diseased birds. These are the main facts learned by the brief investigations of last summer. While we may make certain conjectures as to the modes of transmission of the infectious agent based on these investigations, we are at present unable to go further than this until experiments have been carried forward in other directions.

From our present standpoint I think it safe to assume that the micro-parasite is transmitted from bird to bird without passing through

¹ Bericht ü. d. Veterinärwesen im Königreich Sachsen, 1872, p. 84.

any intermediate host. The microparasite, discharged perhaps in an encysted stage from the sick bird, is taken up with the food and water by others and sets up disease directly.

If the parasites were taken up with insects, for example, we should expect to find the disease diffused through all flocks. But even the limited experience of last summer leads me to believe that certain flocks only are infected and that by uninterrupted transmission the disease becomes perpetuated and diffused among neighboring flocks. The perpetuation of the parasites I am inclined to think is to be sought for in the older turkeys, which carry them in their body, most likely in the digestive tract during the winter. This view is supported by the fact that turkeys not infrequently recover from the disease. This recovery does not mean, however, the destruction of the parasite within the body, for we have now information concerning the persistence of a variety of parasites—bacteria and protozoa—within the body long after recovery.

The evidence which I have presented in this report seems to show that the turkey is infected early in life and that infection does not take place later on. If this be so, the transmission from the old to the young is easily understood. If the susceptibility to infection remained for any length of time we should expect to find the two cæca infected in every case. But in 7 out of 18 cases only one cæcum was found diseased, the other normal. It is true that other explanations of this condition referable to acquired immunity, might be given, but the one cited seems to be the most acceptable.

If the theory that the disease is transmitted more or less directly from old to young should prove to be true, the remedy for such a state of affairs would appear to lie in two directions:

(1) The disease might be allowed to go on and some remedy found which will check it and lead to a cure; or

(2) The diseased flocks might be entirely destroyed and new birds obtained elsewhere, after a thorough cleansing and disinfection of the territory formerly occupied by the flocks.

The latter alternative, though very heroic and severe at first, seems to me the best in the end, especially as we might search in vain for a remedy which will check the disease in the young. Anticipating the investigations somewhat in this direction, I had hoped to determine the distribution of this disease in the country at large by an appeal for the shipment to this Department of diseased turkeys or organs therefrom. Up to the present no material has been received, so that the study of distribution has made no progress.¹ It is evident that if the disease must be weeded out by a destruction of whole flocks it is highly important to find some locality free from such disease from whence new stock may be obtained. To obtain such information is by no means an

¹The cæca of about 30 turkeys from the Washington markets were examined during February of 1895, and found free from disease.

easy task, and may be made still more difficult by the infusion of a certain amount of local pride and self-interest. The disease is, however, readily recognized in most cases, so that anyone who has examined the plates of this article and read certain portions of the text may be able to detect it. The peculiar circular spots on the liver, coupled in later stages of the disease with the presence of yellowish cheesy masses or whitish scars in this organ, are very characteristic and probably peculiar to this affection. Disease of the caeca, unless accompanied with the spots on the liver, should not for the present be regarded as belonging to this affection.

When we come to the subject of treatment some difficulties arise. Though turkeys may appear drooping, unable to keep up with the movements of the flock, and even have diarrhea, the precise nature of the disease is not made clear by these symptoms. Among the 50 turkeys examined many were taken alive as suspicious cases. When killed the protozoan disease was not found. They were suffering from a variety of other affections, the causes of which, mentioned in the order of frequency, were lice, tapeworms, gapeworms, ticks, injury, and a diphtheritic disease of the caeca likely to be mistaken for the genuine protozoan disease. The same treatment applied to such a variety of disorders can hardly be looked upon very hopefully. Still, the subject merits attention. The success which quinine has had in combating malaria leads me to suggest its use to those who are in a position to experiment with this disease. Since the microparasites are embedded in the walls of the caeca and in the liver the remedy to be tried must do more than act locally in the intestines. It must act upon the parasites through the blood, and its effect on the diseased turkey should be carefully watched to note any poisonous action.

The disinfection of the coops and other structures designed to give shelter to turkeys, and other poultry as well, should be carried on as for bacterial diseases until further investigations shall have been made. The following disinfectants are strong enough to kill spores of bacteria, and will probably destroy the various stages of the protozoa:

(a) Corrosive sublimate (mercuric chloride), 1 ounce in about 8 gallons of water (one-tenth of 1 per cent). The water should be put into wooden tubs or barrels and the powdered sublimate added to it. The whole must be allowed to stand for twenty-four hours, so as to give the sublimate an opportunity to become entirely dissolved. Since this solution is poisonous, it should be kept covered up and well guarded. It may be applied with a broom or mop, and used freely on all woodwork. Since it loses its virtue in proportion to the amount of dirt present, all manure and other dirt should be first removed before applying the disinfectant. The manure should be covered with lime.

(b) Chloride of lime, 5 ounces to a gallon of water (4 per cent). This should be applied in the same way.

(c) The following disinfectant is very serviceable. It is not poisonous, but quite corrosive, and care should be taken to protect the eyes and hands from accidental splashing:

	Gallon.
Crude carbolic acid	}
Crude sulphuric acid	$\frac{1}{2}$

These two substances should be mixed in tubs or glass vessels. The sulphuric acid is very slowly added to the carbolic acid. During the mixing a large amount of heat is developed. The disinfecting power of the mixture is heightened if the amount of heat is kept down by placing the tub or glass demijohn containing the carbolic acid in cold water while the sulphuric acid is being added. The resulting mixture is added to water in the ratio of 1 to 20. One gallon of mixed acids will thus furnish 20 gallons of a strong disinfecting solution, having a slightly milky appearance.

(d) Ordinary slaked lime, though it does not possess the disinfecting power of the substances given above, is nevertheless very useful, and should be used more particularly on infected soil.

APPENDIX.

In the following pages is given the history of the turkeys which have been examined up to the time of the completion of this bulletin. It was thought best to include the brief notes of those turkeys affected with maladies other than those of the infectious disease under consideration, to illustrate the existence of such affections and the necessity for further investigations.

Turkey No. 1.—Portions of the liver, cæca, and lungs received in weak alcohol about the middle of October, 1893, from Mr. Cushman. Age of fowl not known. Lungs dark, congested. The wall of the cæcum is fully 5 mm. thick. In the liver are disseminated firm yellowish masses simulating the cheesy matter in tuberculosis. Tissues not in very good condition for microscopic examination. They were, however, placed in absolute alcohol and subsequently cut in paraffin. Sections of lung tissue show all vessels densely packed with red corpuscles. No other changes.

Sections of the thickened wall of cæcum stained in alum carmine, hematoxylin, and eosin, methyl violet, according to Gram, and in Weigert's fibrin stain. The hematoxylin proved to be the best.

The thickening of the wall is due in the main to cell infiltration of the submucous tissue. The entire mucous membrane is sloughed away, and the submucosa presents a ragged appearance along the exposed border. Throughout the section, and more particularly near the serous aspect, there are many distended capillaries filled with blood corpuscles. The nature of the cellular infiltration not recognizable, owing to imperfect hardening. In certain areas there may be seen, however, a peculiar collection of cells having several nuclei and inclosing large vacuole-like spaces. These spaces were subsequently recognized as having been occupied by protozoa. These phagocytes, with several nuclei shaded off into exceedingly large giant cells, over 80μ in diameter, often forming large patches by collecting into groups. They are quite uniformly dotted with nuclei, and contain relatively very few parasites.

Sections of liver tissue showed an extensive substitution of the parenchyma by numerous and greatly dilated blood vessels within newly formed connective tissue.

In such areas only a few islands of liver cells are recognizable. Parasites not seen. Giant cells rare.

Turkey No. 2.—Liver and a portion of the intestine, with cæca attached, sent by Mr. Cushman from Rhode Island on April 2, 1894, and received April 5. Turkey probably a large one.

Post-mortem changes well under way when organs were received. It was, however, not difficult to recognize the disease. Only one cæcum affected. About one-half of the tube, beginning with the blind end, is almost completely occluded with a firm, pale-yellowish exudate having the appearance of concentric layers when cut across, like the rings of a tree. The occlusion is complete at the blind end. The tube, usually only 0.5 to 1 cm. (two-fifths inch) across, is distended to 2 cm. (four-fifths inch). The exudate is firmly attached to the greatly thickened walls. Sections of hardened tissue show that the entire wall of the cæcum, or what is left of it, is densely infiltrated with round cells. The lesions characteristic of the earlier stage of parasitic invasion no longer recognizable.

The liver is beset with a considerable number of circular, slightly depressed areas, which have a pale, mottled appearance. They vary in diameter from 5 to 10 mm. (one-fifth to two-fifths inch). When incised the same appearance is presented within the liver tissue, showing that these circular spots simply represent the surface of roundish masses of tissue which have undergone the same change.

In sections of hardened tissue each disease focus is shown to be the scene of a complex process of destruction. The periphery consists of normal liver tissue, the capillaries of which are distended, and gorged with red corpuscles. Within the diseased focus there is disseminated a peculiar meshwork of amorphous, homogeneous matter which stains but feebly in hematoxylin. Its meshes are large enough to hold one or more tissue cells. In some places it resembles more closely mammalian fibrin in its appearance. Liver cells are either entirely absent or present in isolated numbers where this substance appears. Besides this there are areas of considerable cell infiltration and, scattered about without regularity, giant cells. Parasites are present, but in small numbers.

From the liver bits of tissue were placed in three agar and three gelatine tubes. At the same time bits were placed under the skin of a rabbit and a guinea pig. The cultures remained sterile and the animals well.

Turkey No. 3.—Small specimen; probably 3 or 4 weeks old. Died in the morning of June 6, 1894, on a farm in Berkshire County, Mass. Examined several hours after death. Weather quite cold. In this case both cæca and the liver were involved. In the former the walls were considerably thickened, but there was no exudate within the tube and the mucous membrane appeared intact.

In sections of the cæca hardened in alcohol and in corrosive sublimate the wall was found of variable thickness. In some regions it was nearly normal on one side and considerably enlarged on the opposite side. In others the entire cross section was uniformly thickened. The mucosa is still in position and but slightly altered. The cells of the crypts show extensive mucous metamorphosis. The enlargement of the wall is due mainly to an extensive infiltration of the submucosa and of the circular band of muscular fibers with the protozoa. The muscular fibers are pushed apart into strands, or else entirely obliterated. These microparasites are found throughout the entire section in uniform abundance. They are comparatively rare in the reticular tissue of the mucosa between the crypts. In the fresh contents of the cæca were found coccidia with distinctly double-contoured wall. The long axis measured 15 to 20 μ , the short 10 to 13 μ .

The liver of this bird was dotted with about a dozen circular spots, varying in size, some 7 mm. in diameter. These spots consist in the main of minute yellowish lines embedded in the liver tissue and loosely interlacing to form a circular wheel-like area. They thus differ from some other cases to be recorded in less destruction of liver tissue within the circular area, for the yellowish lines represent necrosis of liver tissue.

In sections of liver hardened in corrosive sublimate and alcohol the foci of disease no longer contained any liver tissue. This is replaced by numerous microparasites surrounded and enveloped in a meshwork of reticulated tissue rich in nuclei. Giant cells are present in moderate numbers.

At the time this young turkey died two others were found dead and were examined. Neither was affected with this disease.

Turkey No. 4.—Has been dead for two days. Decomposition well under way. Obtained from a dealer. Extensive spotted disease of the liver and thickening of walls of caeca with exudation into the tube.

Turkey No. 5 (Farm A).—Probably 8 to 9 weeks old. Received alive August 3. Blood from vein of the skin examined. A few fusiform flagellates seen. Nothing abnormal with the corpuscles. Killed. On the neck a small tick. Mouth, trachea, and lungs normal. The digestive tract normal with exception of the duodenum and the caeca described below. The former shows marked pigmentation in points and striae (villi). In the rectum and cloaca contents pale-yellow, liquid, containing urate spheres in abundance and numerous flagellates.

Both caeca are diseased. They are unusually short, only one-half the length as usually found in turkeys of the same age (Pl. I, fig. 2). Both are distended from one-half to three-fourths of an inch in diameter, and very firm, as if filled up. Vessels on the serous surface injected.

The condition of one of them is shown in Pl. II, fig. 2. The caecum is slit open longitudinally, and the cut surface exposed to view. The lower two-thirds of the tube is completely occluded by a firm exudate. On the cut surface the wall of the caecum is shown as an irregular line, indicating marked thickening along the whole length of the tube. The exudate is pale yellowish-white in color, excepting below, where it is largely made up of a blood clot. The main mass contains centrally an irregular cavity. Above, the tube is filled with small round stones, probably discharged from the gizzard. The other caecum has its wall very much thickened, as shown in Pl. II, fig. 3. The exudate is also present, but not attached to the walls in the alcoholic preparation.

The liver, which is enlarged slightly, is spotted on all surfaces with isolated and confluent areas of a circular outline and pale yellow in color. They rarely exceed 4 mm. (one-sixth inch) in diameter. On closer scrutiny the yellowish color is seen to appear as a network. These disks correspond to masses of diseased tissue within the substance of the liver.

In crushed, fresh tissue are found isolated and agglomerated bodies of a round form, with a homogeneous colorless disk and a single-contoured outline (Pl. V, fig. 1). Near the center of these bodies is seen a small mass of very fine refrangent granules. They are 8 to 10 μ in diameter. One per cent acetic acid will not affect their appearance.

Three cultures on inclined agar were made with a large and two small bits of liver tissue and a large bit of spleen tissue (about the size of a pea). Furthermore, tubes were inoculated with a loop of blood and with a loop driven into the liver tissue. The six tubes remained free from growth.

Turkey No. 6 (Farm B).—Selected because of slightly reduced condition. Cooped for two days. Found dead August 5.

In abdomen some blood-stained serum. Considerable hyperæmia of serous covering of gizzard and of mesenteries. The blind ends of caeca knotted inextricably together, enveloped in a mass of yellow exudate, and attached by means of it to neighboring coils of the intestine and to the abdominal wall. The latter is infiltrated and discolored at this point. After some dissection it was found that only one caecum was primarily diseased. This was filled with an exudate at the blind end for a distance of three-fourths of an inch.

The liver is somewhat enlarged, and on its surface are a small number of pale yellowish areas fully one-half of an inch in diameter. They correspond to masses of similarly affected tissue in the liver.

Sections of liver tissue hardened in alcohol were made through these large pale spots. In these only small fragments of recognizable liver tissue were found interspersed in the diseased focus. Much of this was occupied by a meshwork representing necrosed liver cells fused together. Small necrotic foci were also detected outside of the main focus, embedded in still unchanged tissue. The necrotic meshwork is arranged in spots generally connected, the interspaces being filled up with groups of minute cells and occasional patches of microparasites. Giant cells are common, usually within or on the circumference of the necrotic areas. The microparasites are also inclosed in these cells. The scarcity of these bodies makes it probable that the disease is not longer progressing.

Cultures were made by transferring a loop of heart's blood and a loop which had been forced into the liver through a scorched area to inclined agar.

The blood culture remained sterile. The liver culture contains manifestly several species, only one of which was studied, and found to resemble *B. coli*.

Turkey No. 7.—Received from a dealer the intestines of 16 turkeys for examination. In one of these there was disease limited to one cæcum. (See page 22.)

Turkey No. 8 (Farm C).—Received about August 3 and cooped temporarily. Found dead August 6; alive the day before.

The lesions in this animal are restricted almost entirely to the cæca. In one cæcum the mucosa is beset with about eight peculiar elevations, which have a central yellowish slough and a surrounding infiltrated zone, making the whole crater-like body about three-eighths of an inch in diameter. On the serous surface some of these necroses show as whitish, discolored areas, with injected border.

The other cæcum contains but one ulcer. In the liver several small areas are found which appear necrotic, but whose nature is doubtful.

Transverse sections of a portion of the wall of the cæcum hardened in alcohol were made through one of the ulcers. The mucous membrane had sloughed away over the center of the ulcer, and the submucous tissue and the muscular coat were extensively infiltrated with masses of cells.

Two cultures were made on inclined agar, one with a loop of heart's blood, the other with a bit of liver tissue. The latter remained sterile. From the former two bacilli were isolated, one corresponding to *B. lactis aërogenes*, the other to *B. coli*.

Turkey No. 9 (Farm D).—Brought to laboratory dead. Probably 3 months old. The disease is restricted to the cæca and the liver.

In one cæcum there are three, in the other four, thickenings of the wall, which appear as opaque yellowish spots under the serous covering. Two thickenings are situated near the blind end, the rest near the attached end. From the mucous surface they appear as yellowish spots from one-eighth to three-sixteenths of an inch thick in the center and thinning out from that point.

The liver is beset with large, isolated and confluent roundish patches of a grayish color, mottled with yellowish specks. The same appearance is presented by the cut surface.

In crushed preparations of the liver a few round, uniformly granular bodies, some within what appear to be the remnants of cells, detected.

Sections of liver tissue hardened in alcohol present the usual appearances characteristic of the disease. Sections passing through the circular spots show that a considerable area of such spots consists of the homogeneous meshwork resulting from necrosis and fusion of the liver cells. In some of these, giant cells are situated, singly or in groups, many of them inclosing vacuoles which represent the former seat of microparasites. The regions not occupied by necrotic tissue are largely made up of cells which have a small, round, densely-stained nucleus, enveloped in considerable protoplasm. The nature of these cells is unknown, although it is probable that they correspond to the round cells in the inflammatory processes of mammalia. In the liver tissue beyond the diseased circle isolated foci are present, which are made up mainly of the cells described and some interspersed giant

cells. Throughout the diseased area the various cell forms are undergoing degeneration. This manifests itself by a breaking up of the nuclear substance into stained granules of limited number. This phenomenon is especially frequent in the epithelium of the bile ducts, and more rarely the giant cells themselves show this degeneration.

Sections were also made through one of the thickenings in the wall of the cæca. These showed that the thickening was due in the main to a very extensive cell infiltration of the submucosa and of the muscular portion of the wall. Under the serosa the blood vessels are much dilated, and gorged with red corpuscles. The mucosa is not thickened, but is more or less infiltrated at the base. Much of the epithelium is gone, both of the surface and of the tubules.

The microparasites are poorly preserved, but a little scrutiny shows them to be present in certain regions of the mucosa, submucosa, and rarely in the cell masses between the displaced muscular bundles. In the mucosa and submucosa they have invaded large patches, and here they are ranged side by side and are inclosed in a reticular tissue, each parasite by itself. Giant cells are also present in groups, usually consisting of little else than a mass of parasites enveloped in a nucleated network of protoplasm.

Turkey No. 10 (Farm E).—Probably 8 to 9 weeks old. Supposed to be infected because unthrifty. Cooped for a few days. When killed no lesions found. On the skin were some ticks and lice; in the intestines a few tapeworms.

Turkey No. 11.—From the same flock and in the same condition as No. 10. No lesions found when animal was killed. A few ticks on border of meatus of ear. Lice abundant.

Turkey No. 12 (Farm C).—Probably 3 weeks old. Cooped for a week before it was killed. A few flagellates in blood from veins of skin.

Tapeworms in upper small intestines. In soft contents of cæcum immense numbers of flagellates. No disease of cæcum or liver.

Two cultures made with bits of liver added to agar remained sterile.

Turkey No. 13 (Farm F).—Brought alive, because suspected of being diseased. No lesions discovered on dissection. Many small tapeworms in duodenum. In cæcal contents many flagellates.

Turkey No. 14 (Farm G).—About 3 months old. Taken from a flock August 8 because of lack of strength to keep up with the rest when driven. Indications of diarrhea. Placed in a coop, where it died during the night. Examined next morning.

Slight odor of decomposition. A few small warts on skin of neck. The various organs were found normal, with the following exceptions:

Mucosa of duodenum almost blackish, from intense injection and pigmentation of villi.

Both cæca diseased. The left is slightly distended. On serous aspect two yellowish spots, with markedly injected borders, corresponding to thickenings of the walls near the blind end of tube. The mucous surface of one is smooth; to the other an exudate is attached. Besides the thickening at these spots, the free half of this cæcum is somewhat thickened uniformly.

The right cæcum is very much distended over two-thirds of its length. From the serous surface local thickenings are recognizable, which have a yellowish, mottled appearance. The small intestine is firmly attached to one of these. The disease has, however, not invaded the wall of the latter. The border of these spots is intensely hyperæmic. When the cæcum is slit open its width is three to four times that of the undistended tube, and the thickness of the wall varies from one-eighth to one-half of an inch, being not less than one-eighth of an inch over three-fourths of the entire length. When the brownish feces were washed away the increased local thickenings were found covered with firm exudates, usually attached in but one spot.

Sections were examined of that portion of the cæcal wall which was very much thickened, and to which the contiguous small intestine was inseparably attached by the new growth.

The mucosa of the cæcal portion had sloughed away, while that of the embedded small intestine was intact. The neoplastic tissue between cæcum and intestine was fully 1 cm. (two-fifths inch) thick. Inasmuch as the infiltration probably followed the narrow mesentery between cæcum and intestine the original boundary lines of the cæcal wall are no longer recognizable. The muscular coat of the cæcum may be traced for only a short distance into the neoplasm, when it disappears. Micro parasites were not seen distinctly in the diseased tissue.

The liver is very much enlarged, and dotted everywhere with roundish spots of varying appearance. The majority are from 5 to 12 mm. in diameter, round or slightly oval. The center of each is usually occupied by a group of yellowish dots and the circle is bounded by a narrow yellowish ring. The space of the circle is mottled brownish. Among these spots there are also circles of a completely yellowish color. On the convex surface of the left lobe there is a very firm, ring-like, yellowish mass, cutting like firm cheese.

In crushed preparations of fresh liver tissue from within the brownish circles many giant cells are seen. They consist of a meshwork of protoplasm of a rather coarsely granular character inclosing spheres which appear homogeneous. The giant cells are up to $30\ \mu$ in diameter.

Sections of liver tissue hardened in alcohol and in Foa's solution were also examined. The foci of disease contain necrotic areas in which are numerous giant cells each inclosing a number of microparasites. In some portions there is much cell infiltration in the interlobular tissue around the portal vessels. Among the cells the protozoa are recognizable.

Turkey No. 15 (Farm H).—Obtained alive and killed. About 3 months old. No cæcal or liver disease detected. In duodenum numerous tapeworms. This turkey had probably been injured, for blood extravasation was found under skin of skull.

Turkey No. 16.—About 3 months old. Obtained with No. 14 from the same flock (Farm G). Suffering with diarrhea. After being cooped for two days it was killed August 10. The blood taken from a cutaneous vein of the breast under the wing showed extensive leucocytosis.

The digestive tract is normal, with following exceptions: The mucosa of duodenum is hyperemic. Tapeworms absent. The rectum, i. e., the bowel from the cæca to cloaca, is also hyperemic. It contains yellowish masses made up of microscopic spherical crystals (urates).

Both cæca are affected. Contents of a pasty, brownish (nearly normal) character, containing a considerable number of microparasites. Flagellates not seen.

The disease is manifested in both cæca symmetrically by a thickening of the walls of the distal half of each tube. There are a few yellowish spots noticed under the serous covering of the thickened region. These correspond to spots of maximum thickness. The mucous membrane appears intact. The slight pigmentation noticed on the mucous folds is frequently present in cæca otherwise normal.

In tangential sections of the thickened wall in the fresh condition considerable numbers of round microparasites, of a homogeneous appearance, and about 8 to $10\ \mu$ in diameter are seen. They are not bound to cells, but appear to be displaceable under the cover glass independently.

In transverse sections of one cæcum hardened in corrosive sublimate and alcohol the increase in thickness of the wall was found to be due to hyperplasia of the submucous tissue and cellular infiltration of the muscular coat. The mucosa itself was intact, the epithelium in place. Interspersed in the hyperplastic submucosa are small nests of giant cells, some containing well-defined microparasites, others only vacuoles, somewhat larger than the parasites. The latter are not diffusely scattered through the tissue, but restricted to these and a few other foci in which they exist free in the meshes of the tissue. The masses of cells between the bundles of muscular fibers resemble those of the submucosa and are free from parasites.

The liver is considerably enlarged. The surface is not smooth but slightly roughened. Scattered over the various surfaces there are in all six or seven yellowish-white

homogeneous sclerotic areas which correspond to masses in the liver tissue. In cutting into the liver these foci are found to sheath the hepatic veins. Outside of these sclerotic foci the liver tissue is mottled with gray. In general, the entire liver appears to have passed through a process of sclerosis.

In sections hardened in Foa's solution and in corrosive sublimate the pathological change going on is shown to be essentially a sclerosis following the protozoan infection. Within the disease focus the liver tissue, recognizable as such, is present only in irregular patches of variable extent. The remainder has been replaced by an actively developing connective tissue, still rich in nuclei. Within these areas there is an extensive formation of bile ducts. Parasites were seen in but one spot, where a number of multinucleated cells had ranged themselves around what appeared to be an old thrombus. Here, beyond the encircling giant cells, a group of similar contiguous cells contained a small number of protozoa. The encircling cells themselves also contained a few, and one parasite was recognizable within the thrombus itself. Outside of these spots undergoing repair, the blood vessels are everywhere inclosed in sheaths of new connective tissue very rich in nuclei, encroaching slightly upon the liver tissue itself.

Turkey No. 17 (Farm I).—About 3 months old. Found August 11 in a dying condition in a flock in which a considerable number had died during the past month. The only lesions found were a rather pale condition of the organs. In the small intestine numerous tapeworms. No liver or cæcal disease. In the cæca feces are rather dry, and contain a considerable number of coccidia, varying slightly in size, the largest $32\ \mu$ long and $18\ \mu$ broad.

Turkey No. 18.—From the same flock as No. 17, and quite sick when found. Killed and placed on ice over night.

In this bird no marked lesions of any kind were noted. The duodenum was hyperæmic and contained more or less mucus. The contents of cæca are fluid and contain numerous flagellates. Cultures on agar from blood and liver remain sterile.

Turkey No. 19 (Farm J).—About 3 to 4 months old. Taken August 11 from a large flock, because suspected of being diseased. Died shortly after being caught. Placed in refrigerator over night. In the pleuroperitoneal cavity a dark clot and much blood-stained fluid; ecchymosis of the left abdominal wall. Source of hemorrhage not detected; probably brought on while being caught.

Organs very pale but not diseased, with exception of liver and the right cæcum. In the latter there are two diseased areas. Near the blind end there is a ring-like thickening of the wall, which has a yellowish color from the serous aspect. The other area is covered with a small mass of exudate, and the thickening of the wall beneath it covers an area about one-half of an inch square. The remainder of the cæcum pigmented in spots and lines.

The liver is very much larger than in the normal condition and covered everywhere with circular spots of varying appearance. There are:

(1) Two completely necrotic, yellow masses of cheesy character, one in the base of each lobe.

(2) Circles of a grayish-yellowish color throughout.

(3) Spots of a mottled brownish appearance. The mottling represents distended vessels.

(4) Spots like the preceding with a necrotic mass in the center. These spots vary in size up to a diameter of three-fourths of an inch.

In crushed preparations of the fresh liver the microparasites are distinctly recognizable. Some appear as free granular bodies, others are inclosed, either singly in a cell, or in groups in large cells. These inclosed bodies are usually free from granules and may show a minute nucleus. They measure from 10 to $13\ \mu$ in diameter.

In sections of liver tissue from this case hardened in alcohol the lesions were found to correspond to those in other cases. Sections through a brownish spot showed everywhere marked distension of the vascular channels. These were filled with

corpuscles. The parenchyma has largely disappeared and small cells have taken its place. In a few places of the section microparasites are found collected together in large patches. In these, giant cells are occasionally seen.

Turkey No. 20 (Farm I, see Nos. 17 and 18).—Found in field resting on keel and moving along ground with aid of wings. Obtained August 11. Killed and examined August 12.

In trachea six pairs of gapeworms attached. In cæcum and rectum a yellowish fluid (urates) containing many flagellates. No disease of liver or cæca.

Turkey No. 21 (Farm K).—Picked out of a large flock August 11, 1894, because it was much weaker than the rest. No distinct appearances of disease. When killed the organs were found normal, with the exception of the intestines. A mass of exudate lying adjacent to rectum in abdominal cavity binds together rectum, cæca, and neighboring coils. This mass has a cylindrical shape and is about 2 inches long and three-fourths of an inch at greatest diameter. It consists of spongy yellowish exudate, incased in a smooth, leathery capsule. There is no disease of the mucous or submucous tissue in any part of the intestines. The exudate is evidently the result of a tear in the rectum which permitted the escape of feces into the abdomen. These became subsequently permeated with and encased in fibrin while the tear healed up. Odor of this mass strongly fecal. A bit of liver tissue is placed on inclined agar. The latter remains sterile.

Turkey No. 22.—Large female, over 1 year old. Died on Block Island during the night of August 12. Examined on the farm next day. No evidence of liver or cæcal disease. In abdomen a partially smashed eggshell attached lightly to mesenteries.

Turkey No. 23 (Farm L).—About 3 months old. Received, dead, by express August 14. Probably died two days ago. Slight odor of decomposition.

Duodenum very much discolored by pigmentation. Both cæca considerably distended. The left is normal, the right diseased. Fully one-half to two-thirds of its entire length beginning with the blind end is thickened. There are a number of centers where the thickness is increased. At one of these there has been an outpouring of exudate on the serous membrane. The mucous surface of these local thickenings is covered with firm masses of exudate.

The liver contains one sclerotic focus which has a whitish mottled appearance. It is otherwise normal. Three adult pairs of syngames in the trachea.

In sections of the wall of the greatly enlarged cæcum hardened in alcohol the lesions were of an advanced character. The mucosa was almost entirely sloughed away. Only a few remnants of the slough remained, containing some tubules recognizable as such. The submucous tissue was greatly thickened by cell infiltration, and the muscular coat was divided into sections by cell masses which extended to the serous membrane. Under the latter the blood vessels appeared greatly dilated and filled with corpuscles. The microparasites are still present, but in small numbers. They appear in the neoplastic tissue in small patches inclosed by a fringe of round cells. Giant cells are numerous and arranged in groups. Only in one group were microparasites inclosed.

Turkey No. 24.—Taken August 13 from a flock on Block Island, because it was suspected of being diseased. Killed August 14. No lesions were discovered in the organs. One gapeworm found in the trachea and several minute tapeworms in the intestine.

Turkey No. 25.—Taken from the same flock. When killed disease was found in one of the cæca. (See page 22.)

Turkey No. 26.—About 3 months old. Brought August 15 from a large herd (Farm G), and reported as having been feverish for a few days past. The bird is killed and found in well-nourished condition.

Disease limited to one cæcum and to the liver. Fully one-third of the cæcum has its wall thickened. The thickening is most pronounced in spots, to some of which a

firm, pale-yellowish exudate is attached. A portion of the diseased tube is placed unopened in hardening fluids for further study.

The liver is enlarged to two or three times its normal size, and uniformly permeated with closely set, but usually discrete, spots, varying in size, a few having a diameter of one-fourth of an inch. They are of a yellowish color, this being due to a fine yellowish network, of which the spot is in fact made up. The details are well shown in the photographic reproduction and in the colored drawing. (Pl. III, figs. 1, 2.)

In teased and crushed preparations of the fresh liver a large number of micro-parasites are detected. They appear as round, pale, homogeneous bodies, with a sharp line as a border. They are less refrangent than fat globules, and suggest somewhat the color and refrangibility of myelin or Buhl's bodies as they are found in the alveolar epithelium of the lungs of cattle. Their diameter ranges from 12 to 14 μ . Some are surrounded by a narrow rim of protoplasm, containing one or more nuclei, evidently the remains of a cell.

Transverse sections of the diseased cæcum show a mass of exudate occupying a portion of the lumen. In the exudate are embedded strips of perfectly preserved epithelium. The exudate appears amorphous and contains small foci and even large patches of cell masses, probably originating from the inflammatory processes going on.

The thickening of the wall is due in this case chiefly to infiltration of the sub-mucous tissue. In one portion of the sections the muscular portion of the wall is infiltrated to the serous covering. Parasites are, however, not detected in the sub-mucous or muscular cell masses, but are situated exclusively and in very large numbers in the reticular tissue of the mucosa, between the crypts. In this tissue, as well as in the epithelium of the crypts, numerous mitotic figures are recognizable. Giant cells are also present, but in small numbers. The microparasites are lodged, generally singly, in cavities surrounded by one or more nuclei. In some places few or many may be detected, packed away in a space of the reticular tissue.

The parasites are well brought out in the tissue hardened in corrosive sublimate and alcohol, and stained in hematoxylin and eosin. They appear as round or slightly oval bodies, with a faint reddish tinge of the body and a minute blue, ring-like, central or eccentric body, probably the nucleus. They vary from 6 to 8 μ in diameter.

The lumen of the mucous gland is densely packed with bodies roundish in outline and about 4 μ in diameter. The cell body is stained a homogeneous red with eosin, the nucleus bluish with hematoxylin. (See page 20.)

Sections of the liver were made from material hardened in alcohol and in corrosive sublimate and alcohol. They were stained in various ways, the most satisfactory stain being hematoxylin and eosin, by which the microparasite was most clearly brought out. Methylene blue was also satisfactory in bringing the microparasites into relief.

The sections show within some of the areas indicated by the yellowish spots extensive disappearance of the liver cells proper and the presence of a meshwork or network of a homogeneous substance, which suggests a fibrinous exudate, although it has not the fibrillated structure of mammalian fibrin. This meshwork is not uniformly distributed over the affected area, but appears in foci which are connected with one another. The space left is occupied by numerous parasites and giant cells and an occasional group of liver cells. In other foci the liver cells are replaced by a reticulum containing numerous parasites. Usually but one parasite is contained in a mesh and is closely surrounded by one or more nuclei.

Turkey No. 27.—Taken from another Block Island flock as a suspicious case. About 3 months old.

No lesions found in this bird. In the trachea three gapeworms; in the intestine many large tapeworms.

Turkeys Nos. .8 and .9.—Brought from Fishers Island, New York. No lesions in these cases. Many large tapeworms in the intestine.

Turkeys Nos. 30 and 31 (Farm J).—About 3 to 4 weeks old. Brought because they were not doing well. Killed. Intestine found nearly occluded with small and large tapeworms.

Turkey No. 32 (Farm C).—About 3 months old. Killed August 17. No lesions found. In duodenum several tapeworms. In caecum flagellates.

Turkey No. 33 (Farm B).—About 3 months old. In the blood from a subcutaneous vein some flagellates. No lesions in the internal organs. In duodenum some tape-worms.

Turkey No. 34.—Obtained from Farm K August 18. Died on the way to laboratory.

Bird about 10 weeks old. In body cavity blood-stained fluid. One-half of the left caecum, beginning at the blind end, has its wall thickened. The serous covering is reddened and shows a few yellow patches which correspond to certain specially thickened regions of the wall whose mucous covering is necrosed.

The liver is large and covered everywhere with rather large, round, dark-brownish patches having a narrow, yellowish boundary line. These correspond to diseased foci within the liver.

In fresh liver tissue, crushed or cut in sections, oval bodies 12 to 15 μ in diameter are present in considerable numbers. They appear homogeneous; contents not differentiated. Similar bodies, inclosed in groups of two or three in large cells, are also present. They are homogeneous, round in outline, from 8 to 12 μ in diameter.

In sections of liver tissue hardened in alcohol, when examined with a low power, an immense number of microparasites appear embedded in the tissue. These vary but slightly in size (8 to 9 μ). Throughout the invaded spot the blood vessels, large and small, are irregularly dilated and filled with red corpuscles, among which the parasites are not seen. The tissue between the blood vessels is no longer recognizable as hepatic tissue. In some parts of the section it consists in the main of the round parasites, single or in small groups, each individual or group inclosed in a rim of protoplasm provided with one or more nuclei. There is thus inclosing the parasites an imperfect meshwork present whose origin and nature is not evident. In some places it would appear that the parasites are embedded in the liver cell. The nuclei of such cells have undergone proliferation and some of these proliferated cells have degenerated into groups of minute, deeply-stained granules. The impression conveyed by such cells is that they are giant cells. They differ, however, from those giant cells which appear in a later stage of the disease. The meshwork of tissue which has undergone necrosis is present in but a few small areas.

Turkey No. 35.—From the same flock as No. 34 (and No. 21). Obtained August 18. Died on the way.

This case closely resembles the preceding. One-half the length of both caeca involved. The walls are thickened uniformly and in this portion are some small excrescences easily removed *in toto*, leaving only a slight depression.

Liver spotted on all surfaces as in preceding case. Duodenum blackish from injection and pigmentation. Muscular tissue has a yellowish tinge.

In the trachea one gapeworm, and some roundworms in the duodenum.

Turkey No. 36 (Farm N).—About 3 months old. Obtained, just dead, on August 18.

In this case the liver and caeca are diseased. The liver is spotted on all surfaces. The spots vary in appearance. A few have a mottled, pale-yellowish aspect and are fully 1.5 cm. (three-fifths inch) in diameter. The remainder are round blotches of a mottled, dark-brownish appearance. Many of these have coalesced into small groups.

The wall of the left caecum is very slightly thickened and still smooth. The right is in the same condition. At the middle it has a circular ring-like thickening, about 2 cm. (four-fifths inch) wide, of a yellowish appearance on section. Sections were prepared of this region after hardening the entire caecum in alcohol.

The thickened wall, about 8 mm. in diameter at the thickest portion, consists entirely of neoplastic tissue. The mucous layer is gone and the muscular layer is

no longer recognizable. The bulk of the enlarged wall is composed of small cells, in which are interspersed in round and elongated patches large numbers of multinucleated cells, much smaller in size than the giant cells usually encountered. The cells do not distinctly show the microparasites within their substance except in a few cases, probably owing to the time and manner of hardening.

Turkey No. 37.—Obtained August 18 from Farm O. About 4 weeks old. Died the following night. No lesions found, although intestine contained many tapeworms.

Turkey No. 38.—From the same flock as preceding. About 3 months old. Died during the following night. No lesions. One gapeworm in trachea, and very many small tapeworms in intestine.

Turkey No. 39.—From the same flock. About 9 weeks old. Died the following night. Numerous tapeworms in intestine.

Turkey No. 40.—From the same flock August 18. Cooped until August 20. On that day the bird was resting on its breast with eyes closed. Killed. Many small tapeworms in intestine.

Turkey No. 41.—Probably 8 weeks old. Brought to laboratory August 18, 1894. Cooped temporarily and found dead August 20. Disease in this case limited to both cæca. (See page 22.)

Turkey No. 42.—Obtained August 18 from Farm I. About 3 months old. Suspected of being diseased. When killed no lesions were detected.

Turkey No. 43.—Obtained August 18 from Farm P. Cooped. Found dead August 20. The only discoverable lesions were a puffing out of the tissue below one eye, due to accumulation of a glairy secretion (roup). The same exudate found in the trachea. Agar tubes inoculated with tracheal exudate remained sterile. Numerous tapeworms in intestine.

Turkey No. 44.—Obtained from Farm Q August 18. Many turkeys are reported to have died on this place. Bird about 3 months old. No lesions found when it was killed. Many tapeworms in intestine.

Turkeys Nos. 45, 46, and 47.—Obtained from Farm R August 18 and cooped for several days. When killed nothing abnormal detected. A few tapeworms in the small intestine of No. 45 and No. 47, and many in No. 46. In the semiliquid contents of the cæca of No. 47 very many flagellates.

Turkey No. 48 (Farm S).—Obtained August 18. Cooped until August 21. Bird appears well. When killed a few tapeworms were found in the intestine.

Turkey No. 49.—Received August 18, from a dealer, the intestines of nine turkeys, in only one of these slight traces of cæcal disease. This is manifested by a nodular, roughened condition of the mucosa of one cæcum near the attached end. In all intestines tapeworms present.

Turkey No. 50.—Obtained September 26 from a farm in the District of Columbia. Slight odor of decomposition. The internal organs are free from disease. In the trachea one pair of gapeworms. The head is badly diseased. On the root of the bill a projecting tumor overlapping the nares, which is about two-thirds of an inch in diameter. It is of a soft, medullary character and has a wart-like structure. Besides this large tumor there is a small nodule on the lid of left eye. Right eye completely closed by excrescences on the lid and cheesy masses in conjunctival sac. Cornea opaque. In one nasal passage a large cheesy mass. No liver or cæcal disease.

Turkey No. 51 (Farm B).—Received from Mr. Cushman, Kingston, R. I., October 8, 1894. Died on the way. Turkey quite large; probably 5 months old. Decomposition well advanced. The disease in this case was limited to the cæca, the liver, and the peritoneum. The intestine was covered in places by a firm, yellow pseudomembrane fully 1 mm. thick, and the coils were glued to each other so that they could be separated only after much dissection. The cæca appeared as an irregular mass partly covered with the pseudomembrane. Attempts to dissect out both cæca were fruitless, as they were firmly attached to each other. Only one was diseased, however. This was much enlarged, its walls thickened, and the mucous membrane

beset with large, firm masses of exudate. In the liver were two foci of disease, each about 1 cm. in diameter. One is made up of firm, pale-grayish tissue, probably largely cicatricial; the other is similar in appearance, but has small, yellow, cheesy masses disseminated through it.

Owing to the very advanced stage of decomposition, cultures were not made. It was evident, however, that the cause of death was the septic peritonitis, which was secondary to the cæcal disease.

Turkey No. 52.—Received, dead, with No. 51. Large male bird; probably 5 months old. Very little decomposition.

Head free from disease. Heart and lungs normal. The pleuroperitoneal cavity contains a large amount of blood-stained serum. Serous coat of gizzard very much injected.

Serous coat of proventriculus dotted with elevated yellowish points and lines where the diseased liver lay in contact with it. Spleen small, contains a minute yellow focus; probably also the result of neighboring disease of the liver.

Duodenum, and a portion of the intestine below it, intensely hyperæmic and pigmented. A few small tapeworms present. Pigmentation and hyperæmia gradually become less and disappear near openings of cæca. Below these the intestine is coated with a yellowish liquid, largely composed of urates.

Both cæca contain pigment spots, and the longitudinal folds are pigmented. In one cæcum, near the blind end, there is a thickening of the wall about half an inch across, covered with a brownish-yellow slough on the mucous side. The serous aspect is discolored, in part yellowish. This is the only lesion found in the cæca.

The liver is very large, weighing 304 grams (10.7 ounces). It is extensively dis-eased. The lesions are best described by grouping them under several heads:

(1) Necrosis of the liver tissue is present in both lobes in the form of large and small masses of yellow, firm, cheesy masses (Pl. III, fig. 2). In the left lobe fully one-third of the lobe is converted into a single, yellowish, homogeneous mass, situated at the base or attached portion. The necrosed tissue is sharply defined from the adjoining living tissue by an irregular line. The main branch of the hepatic vein is bounded by the necrosed tissue, and the branches from the dead mass are occluded with pale, friable thrombi. In the right lobe, at the base or attached portion, there are several quite large cheesy masses of very irregular outline embedded in the living tissue and in part visible on the surface. Besides these large masses there are disseminated through the liver numerous smaller masses of cheesy tissue, either singly or in groups. These small foci are usually associated with reparative processes.

(2) Foci which have healed and cicatriced wholly or in part are represented by isolated regions, varying in size and attaining a diameter in one case of three-fourths of an inch. The appearance of these foci varies considerably. They may show as pale pinkish-gray spots penetrated by a network of vessels corresponding to the interlobular markings. Others may be the seat of scars and slight depressions characterized by increased vascularity and a pale pinkish-gray appearance on the liver tissue. Finally, they may be intermingled with small cheesy foci, which are located either centrally in the healed region or in numerous foci around it.

Over the entire surface of the liver patches containing networks of enlarged vessels having a blackish color give the liver an injected appearance. There is nowhere an indication that the disease as such is still going on. The extensive destruction of liver tissue, as well as the repair, appear to be the result of an invasion of protozoa now completely checked.

The false membranes which cover some of the healing spots on the liver and the abundant fluid in the abdominal cavity indicate a secondary infection with bacteria through the necrosed tissue.

A PRELIMINARY INVESTIGATION OF DIPHTHERIA IN FOWLS.

By VERANUS A. MOORE.

[With Pl. VI.]

It is well known that a disease which is characterized by the formation of an exudate on one or more of the mucous membranes of the head is widely distributed among the fowls of this country. Although its existence is of long duration its cause and the character of the exudate have not been satisfactorily determined. In Europe outbreaks of a malady comparable to this in their manifestations have been described as epizootics of fowl diphtheria. There are in this country a few popular articles on diphtheria in fowls, but, generally speaking, the term most frequently employed to designate a disease on the heads of poultry is "roup."¹ The range of morbid anatomy which this term originally indicated is not known, but its present conception is exceedingly broad, including, as shown by the American literature,² nearly, if not all, the noticeable disorders of the heads of poultry.

Writers differ in reference to the nature of this disease. Many poultry raisers consider it a local affection, having its origin in the improper care of the fowls, while others insist that it is a highly contagious disease. The latter class strengthen their position by clinical history, in which they show that fowls which had previously been free from this disease soon became infected after the introduction of one or more "rousy" fowls into their midst. Reports show that it is the usual experience that when the disease is introduced it almost invariably remains in a more or less chronic form for an indefinite period, notwithstanding the adoption of the best known methods of caring for the fowls. It is stated by Bennett that "it is undoubtedly trans-

¹The origin of this term is somewhat obscure, but it is supposed to be a corruption of croup, and its application explained on account of a peculiar hoarseness accompanying the respiration of the affected birds.

²This literature is confined for the greater part to articles in poultry and agricultural papers and journals. They are written by poultry fanciers or farmers, who record their experience with the various diseases of fowls, and, as would be expected, they deal more with the treatment than etiology, and while suggestive are of little value in the study of the cause and nature of the maladies of which they treat.

mitted from the sick to the well fowls by their drinking out of the same vessel, as the discharges from the nostrils of the sick birds contaminate the water as they drink."

The lesions and symptoms attributed to this disease are exceedingly variable. Among the more careful writers we find "roup" defined as "a purulent catarrhal affection of the air passages." The localization of the lesions, however, in the air passages is not universally supported. It is usually stated to affect, to a greater or less extent, the conjunctiva and the mucosa of the mouth, pharynx, and larynx, as well as that of the nares. The first symptoms are said to be "dullness and loss of appetite, discharge from the nostrils, loud breathing, and a dry cough." This is followed by the appearance of an exudate on the mucosa of the nares, which extends in all directions, often covering the eyes with a cheesy substance. The secretions are said to occasionally accumulate within the sides of the face, causing them to swell to an extensive degree. The appetite is either very poor, or ravenous, in which case the food is not digested. Emaciation follows and death may occur in from three to eight days, or, as is usually the case, the disease becomes chronic and the fowls eventually recover.

The economic importance of fowl diphtheria appears to be greater than that of any of the other poultry diseases. There are no statistics, however, by which the total amount of loss from this cause can be even approximately obtained, but the numerous reports of its occurrence are sufficient to show that it is very large. Unlike a rapidly fatal disease such as cholera, the loss is not confined to the fowls which die, but in addition it includes a heavy shrinkage in the poultry products, due to the chronic course of the disease in large numbers of fowls which eventually recover. The small value of the individual and the fact that only a small percentage of those affected die has evidently caused the importance of the disease to be underestimated. It is evident, however, that the frequency of its occurrence and its wide geographical distribution render this affection a serious obstacle to the poultry industry¹ of this country. In addition to the usual chronic form, there are, if reports are true, frequent epizootics in which thousands of dollars' worth of poultry are destroyed.

In addition to the theory of contagion, there are many conditions mentioned in poultry literature under which this disease may be expected to occur. Thus, it is affirmed that fowls exposed to a draft at night will ordinarily become affected. The nature of the food, ventilation, and cleanliness are also considered important factors in producing or preventing this malady. The exudates are described as both catarrhal and diphtheritic, but the description of the lesions is exceedingly indefinite.

¹ The economic importance of this industry is little understood. The Eleventh Census shows that in 1890 there were in the United States 258,472,155 barnyard fowls (exclusive of ducks, turkeys, and geese), and that the egg product for 1889 was 817,211,146 dozens with a money value of \$98,000,000.

The foregoing résumé of the present knowledge of the disease generally designated as "roup" is important in showing the various conditions recognized and included under that term. There are other so-called fowl diseases, such as influenza and distemper, reported to be characterized by somewhat similar symptoms and lesions. Their descriptions are so indefinite, however, that it is impossible to differentiate them from certain forms of the disease more frequently called "roup," but which, on account of the nature of the lesions, will be considered as diphtheria in this preliminary report.

I.

During the past year numerous reports of epizootics among poultry¹ have been received, and several sick and dead fowls have been sent to this laboratory and to the experiment station of this Bureau for examination and diagnosis. Certain of the sick fowls improved rapidly and soon fully recovered. Others were dead and far advanced in post-mortem changes when received, while a few were in a condition more suitable for bacteriological and pathological study.

In all, about 36 fowls came under observation. Several of them subsequently recovered; a few died with tumors; in one case death was undoubtedly due to a traumatism;² 2 died of fowl cholera, and the remainder, 18 in number, were affected with somewhat similar lesions of the mucous membranes of the head, although they came from reported epizootics of cholera, diphtheria, and "roup." The examination of these fowls revealed the interesting fact that the lesions corresponded very closely to those described by Loeffler,³ Klammer,⁴ Babes and Puscariu,⁵ Eberlin,⁶ Loir and Ducloux,⁷ and others, as diphtheria in pigeons, fowls, and other birds.

As the disease was not studied in the field it is impossible to define the conditions under which it occurred, or to estimate the full extent of the loss it occasioned. The fowls examined and here reported came from five different flocks, in some of which there is a history of contagion. Unfortunately, the fowls sent to us were usually among the last to be affected.

The methods employed have varied according to the exigencies of the

¹ In addition to the fowl disease, a similar affection of pigeons, more particularly of young squabs, was reported from Waynesboro, Pa., and several of the affected birds were sent to this laboratory for examination. The lesions found in these birds resemble somewhat closely those found in the mouths of fowls.

² It is not uncommon to find pieces of wire or nails in the crop and proventriculus. In one case, two eight-penny nails and a piece of wire about 2 inches long were found piercing the walls of the proventriculus and penetrating the liver and lungs.

³ Mittheilungen aus dem Kaiserlichen Gesundheitsamte, Bd. II (1884), S. 421.

⁴ Berliner thierärzt. Wochenschrift, 1890, No. 18, S. 138.

⁵ Zeitschrift f. Hygiene, Bd. VIII (1890), S. 374.

⁶ Monatshefte f. Thierheilkunde, Bd. V (1894), S. 433.

⁷ Ann. de l'Inst. Pasteur, Tome VIII (1894), p. 599.

different cases. In order, therefore, to present the facts obtained in these preliminary studies, it seems best to record somewhat briefly the history of each outbreak, so far as determined, together with the notes on the individual fowls.

OUTBREAKS.

I.

Late in December, 1893, Mr. W., of Middleport, N. Y., sent several adult fowls¹ affected with "roup" to the experiment station of this Bureau. Mr. W. stated that he had lost a considerable number of fowls from this disease during the past two or three years. He believed that the disease was introduced into his flock by the purchase of some choice birds about three years before. Since its first appearance it had continued to exist in his flock, usually running a chronic course, but occasionally breaking out in a more acute and destructive form. He had tried many methods of treatment without success.

The majority of the fowls sent by Mr. W. appeared to be well upon their arrival at the experiment station. A few of them were killed for examination and found to be normal throughout. Five fowls, however, were suffering with a slight or more extensive exudate on the nasal mucosa, or conjunctiva. These were carefully watched and examined from time to time with the results indicated in the appended notes.

Fowl No. 1.—This fowl was suffering from an exudate in the left eye. December 25 Dr. Smith made several cultures from this exudate. From these two nonpathogenic bacilli were isolated.

December 28: A portion of the exudate was removed from the eye of fowl No. 1 and inserted beneath the skin, over the abdomen, of a rabbit. On the following day there was considerable swelling at the point of inoculation. This continued for several days, when it subsided. The rabbit died February 10, 1894. It was very much emaciated, but no bacteria were found in the organs, either in cover-glass preparations or in cultures.

January 25, 1894: Fowl No 1 was found dead; very much emaciated. The left eye was covered with a thin layer of a friable, yellowish exudate, composed of broken-down cells and bacteria. No fibrin discovered. The nictitating membrane thickened; cornea opalescent. The mucosa of the nares, mouth, and pharynx were apparently normal. No lesions were found in the internal organs. Culture media inoculated with small pieces of the liver remained clear.

Two white mice were inoculated subcutaneously with bits of the exudate from the eye. They died on the fourth and sixth days, respectively, after the inoculation.

From the organs of the mouse which died on the sixth day a nonmotile bacillus, resembling morphologically and in its cultural characters the bacillus of rabbit septicæmia, was isolated. Experimental animals inoculated with small quantities of culture remained well. This bacillus was carefully studied and retained in subcultures until the fall of 1894, when a rabbit inoculated intravenously with a moderate quantity of pure culture died on the second day with lesions similar to those produced by the slightly attenuated bacillus of rabbit septicæmia.

¹The term fowl is used in this article in its restricted sense, referring to *Gallus domesticus* only. This disease, however, is said to affect several other species.

The mouse which died on the fourth day exhibited an enlarged and discolored spleen. The other organs were normal in appearance. A cover-glass preparation showed it to contain a large number of bacilli. Tubes of agar and bouillon inoculated with bits of the spleen pulp developed into pure cultures of a motile bacillus, which was subsequently found to be possessed of marked pathogenic properties. Although fatal to such a large number of experimental animals, experiments to produce a disease in fowls similar to the one from which it was obtained gave negative results.

Notwithstanding the marked pathogenesis of this organism, its cultural and physiological manifestations showed it to be closely related to *Bacillus coli communis*,¹ though differing from it in several important particulars.

Fowl No. 2.—January 4, 1894: This fowl appeared to be well excepting a protuberance beneath the left eye. In order to examine this thoroughly the fowl was killed with chloroform. The autopsy showed much emaciation. The protuberance consisted of an encysted tumor lying immediately beneath the conjunctiva. It was about 2 cm. long and about one-half as thick. Upon section it was found to contain a yellowish, caseous substance, which emitted a peculiar, penetrating, and repulsive odor. The nasal duct was clear. The eye itself was not visibly affected. The liver was unusually fatty. In the left lung were a few hepatized areas. Other organs appeared to be normal.

Cover-glass preparations made from the caseous substance contained many bacteria. Those from the other organs showed no microorganisms. From the caseous substance a large number of cultures were made in agar, bouillon, glycerin-agar,

¹On account of the importance of considering all bacteria possessed of pathogenic properties, of however slight degree of virulence, the more significant characters and properties of this bacillus are appended.

Morphology.—A small, actively motile bacillus, varying in length from 1.2 to 2 μ , ends rounded. It is appreciably larger in bouillon than in agar cultures. Flagella easily demonstrated. Stains readily with the ordinary aniline dyes.

Culture characters.—On agar it develops in twenty-four hours into a moderately vigorous, moist-appearing, glistening growth of a neutral grayish color. The growth is not viscid. When isolated the colonies are round, slightly convex, varying from 1 to 3 mm. in diameter. In gelatin the growth is very feeble in stick cultures. After forty-eight to seventy-two hours a delicate grayish growth appears along the needle track. The colonies in plate cultures appear on the second day as minute grayish points. On potato the day after inoculation at 36° C. a light, lemon-yellow colored growth appears. At the end of from four to six days the growth has increased slightly in quantity; the color is somewhat darker. When cultivated in peptonized bouillon the fluid becomes heavily clouded in twenty-four hours. After about three days a thin, friable, grayish membrane forms over the surface of the liquid. The reaction, which at first is faintly alkaline, becomes slightly acid, but subsequently strongly alkaline. It does not grow in acid bouillon.

The casein of milk is precipitated in from two to three days. It is faintly acid in reaction. In about seven days the casein becomes firm and covered with a clear serum.

Its action upon sugars is very constant. Dextrose and lactose are fermented with the formation of gas. In both cases 46 per cent of the capacity of the closed branch of the fermentation tube is filled with gas. The proportion of CO₂ to the H in the gas varies slightly with the two sugars; in the dextrose it is 3:11, in the lactose 4:11. The production of gas is completed in from three to four days. Fermentation tubes containing saccharose bouillon become uniformly clouded throughout, strongly alkaline in reaction, but no gas is formed.

A marked indol reaction was obtained.

Pathogenesis.—When isolated this organism was fatal to white mice in from twenty-four to forty-eight hours when inoculated subcutaneously with from one to

gelatin, blood serum, bouillon containing glucose in fermentation tubes, and anaërobic-agar cultures. From these two aërobic bacilli and one streptococcus were isolated, all of which were harmless for experimental animals.

Fowls Nos. 3, 4, and 5.—When received these fowls exhibited a very slight quantity of a mucus-like exudate in the nasal cavities. They were thought at the time to be the best cases for an experiment to test the contagiousness of the disease, and very soon after their arrival they were penned with six healthy fowls.

February 21, 1894: About seven weeks later, rabbit No. 144 was inoculated subcutaneously with a loop of the nasal discharge of fowl No. 3. February 23 the rabbit was found dead. The autopsy showed a slight purulent infiltration into the subcutaneous tissues at the point of inoculation. The spleen was slightly enlarged and of a dark, purplish color. The liver was hyperemic, and the cortex of the kidneys deeply reddened, due to injected blood vessels. There were several punctiform hemorrhages beneath the serosa of ileum and lower colon. The lungs were markedly emphysematous. Stained cover glass preparations made from the blood, spleen, and liver showed innumerable short rod-shaped bacteria with ends rounded, usually exhibiting light or unstained centers. Pure cultures of this bacillus were obtained from the spleen and blood.

February 24: A second rabbit (No. 145) was inoculated subcutaneously with the nasal discharge. It was found dead at 4 p. m. February 25. The autopsy and bacteriological examination revealed the same condition and results as those described for rabbit No. 144.

Fowl No. 3.—February 25: This fowl was found dead. It was very much emaciated. The nasal cavities were occluded with a thick muco-purulent substance. At the external nares it had a yellowish color and was dried down into a firm, crust-like mass. Throughout the nasal passages to the posterior opening the substance was lighter in color and very viscid. The eyes were not affected. The internal organs appeared to be normal. Tubes of culture media inoculated from the liver and blood remained clear. The fact that two rabbits had been recently inoculated from the nasal discharge with like fatal results precluded the necessity of further inocula-

two drops of a pure bouillon culture. The lesions were characterized by œdema about the place of injection, and an enlarged and discolored spleen. Gray mice remained well after a similar injection.

A short series of inoculations were made in white mice, and gray mice succumbed to the inoculation with a culture from the spleen of the second white mouse that died. The lesion found was a much enlarged spleen as in white mice.

A guinea-pig inoculated in the abdominal cavity with 0.3 cc. of a bouillon culture was found dead on the following morning. The abdominal organs were covered with a grayish exudate, which was in the form of shreds over the intestines. A few ecchymoses beneath the pleura and peritoneum. The peritoneal exudate contained innumerable bacteria.

Rabbits are not susceptible to subcutaneous or small intravenous injections of pure cultures.

A fowl inoculated in the wing vein with 0.3 cc. of a bouillon culture died on the second day. The organs contained very few bacilli. Tubes of culture media inoculated with bits of the liver developed into pure cultures of this bacillus.

A fowl inoculated subcutaneously with 0.6 cc. of a bouillon culture died in forty-four days. No bacteria were found in the organs and no lesions were discovered. The cause of death is questionable.

A large number of inoculations were made beneath the conjunctiva of fowls, but no lesions were produced. Loops of the growth from agar cultures were rubbed over the conjunctiva and into the nasal cavities with negative results.

Four fowls were fed with 240 cc. of bouillon cultures of this bacillus mixed with a little mill food. They remained perfectly well.

tions at this time. The bacillus obtained from rabbits Nos. 144 and 145 was exceedingly abundant in the nasal discharge, and especially so in preparations made from scrapings of the nasal mucosa.

Fowl No. 4.—This fowl had presented external appearances similar to those of fowl No. 3.

February 24: Rabbit No. 97 was inoculated subcutaneously with a loop of the nasal discharge. It was found dead on the following morning. The examination showed its organs to contain innumerable bacteria identical with those found in rabbits inoculated from fowl No. 3.

Fowl No. 5.—Like Nos. 3 and 4, this fowl suffered apparently from a nasal catarrh. The eyes and mouth were normal.

February 28: Rabbit No. 149 was inoculated with the nasal discharge. The rabbit died in less than twenty-four hours from septicæmia, the same as the rabbits inoculated from fowls Nos. 3 and 4.

Fowls' Nos. 4 and 5 died soon after the inoculations into rabbits were made. They were not brought to the laboratory, but examined at the experiment station by Dr. F. L. Kilborne, who reported them to be much emaciated and the nasal cavities filled with a muco-purulent substance. The bacteria obtained in pure cultures from the rabbits inoculated from fowls Nos. 3, 4, and 5 were carefully studied and compared and found to be identical in morphology and cultural characters and to resemble the bacillus of rabbit septicæmia. The bacillus obtained from fowl No. 1 was not distinguishable from it except in its degree of virulence. ^{*M.H.P.}

The six fowls penned with fowls Nos. 3, 4, and 5 from the latter part of December, 1893, until the middle of February, 1894, remained perfectly well. The disease in the affected ones remained confined to the nasal cavities. In this experiment all the fowls drank from a small basin, and their food was kept in a dish arranged so that they were all compelled to eat from it. The disease was not contracted by the exposed fowls.

II.

In October, 1894, four fowls were received from Mr. W., of Newbern, N. C. In an accompanying letter he stated that they were suffering from a disease which was called cholera, and which had killed hundreds of fowls about Newbern during the summer and early fall.

All of the fowls (Nos. 65, 66, 67, and 68) were adults of a mixed breed. Upon inspection two of them were found to be apparently well and the other two were suffering from an exudate over the conjunctiva of one eye and the mucosa of the nasal passages. As this disease was reported to be contagious it was thought that the apparently well fowls (Nos. 66 and 67) had simply been exposed, and consequently they were kept for a considerable time waiting for the disease to develop. They were subsequently chloroformed and carefully examined, but no lesions were found in either case, and culture media inoculated with pieces of the organs remained clear.

Fowl No. 65 was chloroformed soon after its arrival and carefully examined. It was much emaciated. The left eye was covered with a thick yellowish exudate, which was somewhat elastic. The microscopic examination showed small and large round cells and bacteria. A fibrin-like substance was present in moderate quantities. The exudate was firmly attached to the cornea but not to the conjunctiva covering the inside of the lids. The mucosa of the nares and mouth appeared to be normal. The intestines were slightly reddened, due to injection of blood vessels. They contained a large number of roundworms and several tapeworms attached to the

mucosa. In the intestinal wall were several nodules.¹ The other organs appeared to be normal.

Tubes of culture media inoculated with the liver remained clear. A tube of agar inoculated with the exudate from the eye developed into a pure culture of a motile bacillus belonging to the colon group of bacteria.

Fowl No. 50 was inoculated in both eyes with bits of the exudate from the eye of fowl No. 65. The mucosa of the conjunctiva over the lower lids were scraped and the exudate thoroughly rubbed into the irritated surface. The inoculation wound healed rapidly and the eyes subsequently remained well.

Fowl No. 68 was chloroformed and examined November 9. Fowl very much emaciated. The left eye was covered with a cheesy, yellowish, rather firm exudate, which formed a cap over the eye and which was easily removed. It emitted a strong putrid odor. The eye was much flattened and the cornea had an opalescent appearance. The posterior portion of the eye was normal. The saucer-shaped exudate was loosely attached to the conjunctiva over the lids and cornea. There was a small amount of firm cheesy exudate in the nasal cavities. The mucosa of the mouth and pharynx was normal in appearance. The liver, spleen, kidneys, intestines, and lungs also appeared to be normal. Although the fowl was emaciated, it is highly probable that the exudate in the eye would soon have sloughed and that recovery would have followed. The appearance of the nasal mucosa indicated that the cheesy substance in the nasal passages had come from the eye, having coagulated after passing through the lachrymal duct. The disease appeared to be localized in the left eye.

Fowls Nos. 54 and 55 were inoculated by scraping the mucosa of the conjunctiva and pharynx and thoroughly rubbing the irritated surface with the exudate from the eye of fowl No. 68. The inoculation wound healed and the fowls remained well. About ten days later they were reinoculated in a similar manner with the growth from an agar culture made from the eye of fowl No. 68. No lesions were produced.

III.

In the fall of 1894 several chickens suffering from this disease were examined from the flock belonging to Mr. T., who resided near Alexandria, Va. The insidious nature of certain forms of this malady is well illustrated by this outbreak. Several healthy young chickens were desired for experimental purposes, and Mr. T. reported that he possessed some very thrifty chickens of the desired age, and his account of the history of his fowls showed that the place had been free from fowl diseases for several years. Nine of the chickens were secured and placed on the experiment station. A few days later two of them were brought to the laboratory, where, upon inspection prior to their use, they were found to be suffering with an exudate which covered the greater part of the mucosa of the mouth and pharynx. The other seven chickens were examined by Dr. Schroeder, director of the station, who found a few of them to be similarly affected. The remainder appeared to be well, but were subsequently attacked. Some of these were allowed to die, and others were killed in the earlier stages of the disease for exam-

¹ During the summer of 1894 numerous fowls were found to be affected with a disease of the intestines, due to the presence of tapeworms. This disease is especially interesting, owing to the resemblance of its lesions to those of tuberculosis. I have described it somewhat fully in an article entitled "A Nodular Taeniasis of Chickens," to appear in a forthcoming report of this Bureau.

ination. Mr. T. states that later in the fall he lost a few chickens from this disease, and a large number were affected which recovered.

Fowl No. 60 was brought to the laboratory October 17. On the following day it was found sitting in a crouched position, feathers ruffled, and the head drawn close to the body. It breathed through the mouth. The eyes were usually closed; no exudate on the conjunctiva; temperature 108° F. The symptoms apparently improved during the next few days, but on the morning of October 22 it was found dead. This chicken was about 2 months old and was much emaciated. On the lower side of the cornea of the right eye there was an area about 2 by 1 mm. covered with a grayish exudate loosely attached to the cornea. The mucosa over the entire left side of the mouth was covered with a yellowish exudate one-fourth to 2½ mm. in thickness. It was easily removed and exceedingly friable. The mucosa of the pharynx, larynx, and nares was normal. The internal organs appeared to be normal. Cultures made from the exudate, liver, and kidneys contained a variety of bacteria, which, however, were not isolated and studied in pure culture.

Fowl No. 59 was received at the laboratory at the same time, and it manifested symptoms similar to those described for No. 60. After a few days the symptoms showed a general improvement, which continued until October 20, when it was chloroformed for examination.

The autopsy showed the fowl to be very much emaciated. The mucosa of the nares and the conjunctiva was normal. On the floor of the mouth, on each side of the tongue and extending back on either side of the larynx, there was a band of grayish exudate about 2 mm. in width, firmly adhering to the mucosa. In microscopic section the exudate was found to be composed for the greater part of round cells and bacteria. (See Pl. VI, fig. 3.)

The internal organs were normal in appearance. Tubes of agar inoculated with bits of the liver and kidney developed into cultures containing several forms of bacteria, including a micrococcus frequently encountered and *Bacillus coli communis*.

A guinea-pig was inoculated subcutaneously with a small piece of the exudate, including subjacent muscle. A small swelling developed at the place of inoculation. Five days after inoculation the guinea-pig was chloroformed and examined. At the place of injection the subcutis was infiltrated with a pasty, purulent substance over an area of 5 by 3 cm. The adjacent muscle necrosed. Thoracic and abdominal organs normal in appearance. No bacteria were found in cover-glass preparations and subsequently in cultures made from the organs. The pus in the local abscess contained innumerable bacteria, apparently of the same species. Bouillon and agar inoculated from the infiltrated substance developed into pure cultures of a nonmotile bacillus, which was carefully studied and found to be similar in its pathogenesis and cultural characters to that of slightly attenuated rabbit septicæmia.

Fowl No. 69.—In the latter part of October this fowl was attacked, and a thick exudate formed on the larynx. November 2 it was chloroformed. The eyes were covered with exudate which agglutinated the lids. Upon opening the lids the exudate was easily removed from the conjunctiva covering the lids, but it was firmly attached to the cornea. The exudate formed saucer-shaped caps which covered the eyes, being 2 mm. thick over the cornea, gradually thinning out to a sharp margin. About the margin of the firm exudate was a small quantity of partially coagulated mucous substance. The mucosa of nares normal. On both the anterior and posterior sides of the laryngeal opening there was an area about 3 mm. in width covered with a grayish exudate adhering firmly to the mucosa. It was removed with difficulty, leaving a raw surface. The kidneys were mottled with grayish lines, due to large quantities of urates in certain of the tubules. Other organs appeared to be normal.

November 2: Rabbit No. 215 was inoculated subcutaneously with a small quantity of the coagulated mucus from the eyes,

November 5: Rabbit found dead this morning. At the point of inoculation the subcutis was infiltrated with a purulent substance over an area 2 by 5 cm. The pleura over the lungs and parietes was covered with a thin cellular exudate. Lungs hyperemic. Spleen much enlarged and dark colored. Kidneys hyperemic. The liver mottled with areas covered with a delicate grayish exudate. The mucosa of the lower colon sprinkled with punctiform hemorrhages. Cover-glass preparations from the organs contained innumerable short bacilli with ends rounded. Polar-stain not marked.

Rabbit No. 225 was inoculated with 0.3 cc. of a bouillon culture obtained from the spleen of rabbit No. 215. It was found dead on the fourth day with lesions similar to those found in rabbit No. 215. Stained cover-glass preparations made from the liver and spleen contained innumerable polar-stained bacteria. Pure cultures of this organism were obtained from the liver. A guinea-pig inoculated into the abdominal cavity with 0.3 cc. of a bouillon culture from rabbit 215 died within twenty four hours. A subcutaneous inoculation with a similar quantity produced death in six days.

Fowl No. 70 was received at the same time as No. 69 and chloroformed for examination. Much emaciated. The left eye was closed, the lids agglutinated together; the eye was covered with a yellowish cheesy exudate adhering quite firmly to the cornea. It gave off a disagreeable odor. The exudate had sloughed from the conjunctiva of the lids. In the nasal passages there was a small amount of cheesy exudate. Mucosa of mouth normal. The internal organs appeared to be normal. No cultures were made.

Fowl No. 71 was found dead November 5. This fowl was much emaciated. The left conjunctival sac contained considerable semiliquid substance. The mucosa was slightly reddened and the cornea had an opalescent appearance. No other lesions were found. Cultures made from the exudate in the eye contained several forms of micrococci and a short nonmotile bacillus.

Rabbit No. 223 inoculated subcutaneously with the liquid from the eye.

November 12 the rabbit was found dead. The autopsy showed extensive purulent infiltration into the subcutaneous tissue about the point of inoculation. The thoracic and abdominal organs were covered with a thin grayish exudate composed of cells and bacteria. Spleen enlarged; pulp soft. No bacteria were found in cover-glass preparations from the organs, but in those from the exudate there were innumerable short bacilli with ends rounded, frequently exhibiting a polar-stain. Pure cultures of this bacillus were obtained from the spleen and liver.

November 14: Fowl No. 64 was inoculated in the conjunctiva with 0.2 cc. of a bouillon culture of the bacillus obtained from fowl No. 71. On the following day there was much swelling of the eyelid. The swelling increased, with accompanying reddening, for about two days, when it began to subside and finally entirely disappeared. The fowl continued well.

November 14: Fowl No. 65 (previously inoculated on the mucosa of head) was inoculated in the wing vein with 0.3 cc. of a fresh bouillon culture of this bacillus. The fowl remained well.

Two small chickens were inoculated beneath the mucosa of the mouth and also the conjunctiva with several drops of a pure culture of this bacillus without producing any appreciable effect.

Fowl No. 72 was found dead November 7. It was much emaciated. A thin grayish substance covered the cornea from which a nonpathogenic bacillus was isolated. No lesions could be found, and culture media inoculated from the organs remained clear.

Fowl No. 73 was found dead November 9. It was much emaciated. The conjunctiva and mucosa of the nares were normal. The roof of the mouth and the pharynx were covered with a thick grayish exudate, which was removed with difficulty, leaving a raw surface. The entire tongue was surrounded with a thick exudate, which

was easily removed. The examination of sections showed a cell infiltration extending through the mucosa into the subjacent muscles. No other lesions were found. On account of a scarcity of rabbits no inoculations were made from this fowl.

Fowl No. 75 was found dead November 22. Fowl considerably emaciated. The left eye was covered with a thick necrotic exudate, which was easily removed from both the eye and conjunctiva of the lid. The exudate extended through the nasal duct into the nares. In the posterior nares the exudate was bordered by a layer of a grayish, mucus-like substance. Small areas on the roof of the mouth and about the glottis were covered with a grayish, firmly-adhering exudate, which left a raw surface upon removal. No other lesion discovered.

November 22: Rabbit No. 226 inoculated subcutaneously with the mucus-like substance from the nares.

November 23: Rabbit found dead this morning. Owing to the large amount of exudate injected beneath the skin the local lesion was unusually severe. The abdominal viscera were hyperæmic. Cover-glass preparations made from the organs contained many elongated bacteria. A pure culture of a nonmotile bacillus was obtained from the liver. A rabbit inoculated subcutaneously with 0.3 cc. of this culture died of septicæmia in less than twenty-four hours. This bacillus was identical in its morphology and cultural characters with those obtained from fowls Nos. 59, 69, and 71.

IV.

In October, 1894, a severe epizootic was reported to have occurred among fowls belonging to Mr. P., residing on Bennings road, in the District of Columbia. Mr. P. stated that a considerable number of his fowls had died from what he thought to be diphtheria, on account of the grayish membrane observed on the mucosa of the mouth and pharynx. Unfortunately, he did not notify us of this disease until it was nearly over, so that we had an opportunity of examining only the three last fowls affected. Two of these were not received until some time after death, and the other died on the third day after its arrival. The results of these examinations are appended:

Fowl No. 58.—This fowl was received at the experiment station October 9. It was found dead on the morning of October 12. Fowl very much emaciated. The mucosa of nares, mouth, and pharynx appeared to be normal. No lesions were found in the internal organs excepting a few nodules due to certain forms of tapeworms in the intestine. Cover-glass preparations of the blood and liver showed no bacteria. In those from the kidney a very few short bacilli with rounded ends were observed. Tubes of bouillon were inoculated with bits of the blood, liver, and kidney. The tubes inoculated with the blood and liver developed into impure cultures of ordinary saprophytic bacteria. The culture from the kidney contained a short motile bacillus belonging to the colon group.

Fowl No. 61.—A small chicken, about 10 weeks old. It had been dead about twenty-four hours when received. Much emaciated. Along the cleft in the hard palate there was a narrow strip covered with a thick grayish exudate, which was firmly adherent to the mucosa. On the posterior surface of the pharynx and also about the glottis were small areas covered with a similar exudate. The left eye was covered with a firm, yellowish mass of necrotic exudate, which was easily removed. The color of the internal organs was slightly altered, due to post-mortem changes; otherwise they appeared to be normal.

A guinea-pig was inoculated subcutaneously with a bit of the exudate, including the subjacent tissue from the roof of the mouth. A slight swelling occurred at the point of inoculation. Four days after the inoculation the guinea-pig was chloro-

formed and culture media inoculated from the local lesion. These developed into pure cultures of a motile bacillus belonging to the colon group.

Fowl No. 62.—This fowl was received at the same time as No. 61. The lesions were confined to the nasal passages, which were occluded with a yellowish cheesy substance, and the left eye, which was covered with a quite firm, easily removable necrotic exudate. The conjunctiva of the lids were not appreciably affected. The cornea was opaque.

A guinea-pig was inoculated with a portion of the exudate from the nasal cavities. It was chloroformed six days later for examination. The subcutaneous tissue about the point of inoculation was infiltrated with a purulent substance over an area of about 1 cm. in diameter. No other lesions. From the local lesions pure cultures of a motile bacillus belonging to the colon group were obtained.

V.

This outbreak occurred among the fowls belonging to Mr. H., who resides at Brookland, D. C. He states that he had about 75 fowls, all of which were in excellent condition up to the purchase of a cock, which was subsequently found to be diseased. A large number of his fowls became infected. They usually recovered, although several of the badly affected ones were killed. Late in October Mr. H. brought a hen which was suffering from the disease to this laboratory. This was one of the last of his fowls to be attacked. At the time it came under my observation the disease was confined to the left eye and an area of firmly attached exudate on the roof of the mouth. This fowl was kept under observation for several weeks, and cultures and animal inoculations were made from the exudate as indicated in the appended notes.

Fowl No. 63.—Received October 20. The left eye was considerably swollen, the two lids being agglutinated together. The cornea had an opalescent appearance. Respiration apparently normal. Temperature 107° F. Fowl sits with the head drawn close to the body. Tubes of agar and bouillon inoculated with the exudate developed into pure cultures of a nonpathogenic micrococcus.

A white mouse was inoculated subcutaneously with a loop of the exudate from the eye. It remained well for about three weeks, when it died. As it was badly decomposed when examined no cultures were made.

Fowl No. 64 was again inoculated in both eyes with the exudate from the eye of this fowl. The exudate was thoroughly rubbed on the conjunctiva. Four days later the inoculation was repeated. The eyes remained apparently perfectly well.

October 22: A guinea-pig was inoculated into the abdominal cavity with 0.4 cc. of a bouillon culture from the eye exudate. It remained well.

October 24: The left eye of fowl No. 63 was covered with a quite firm, nearly white exudate, which causes it to protrude considerably. The lids were firmly agglutinated together, being separated with much difficulty. The lid was lined with a thick, quite firm, whitish exudate. Nictitating membrane much thickened. There were several areas of a grayish exudate in the roof of the mouth. Cultures in agar and bouillon made from the eye contained two species of micrococci. The growth in the agar consisted of a few colonies only.

A white mouse inoculated with the exudate was found dead on the third day. From the liver a culture of a micrococcus was obtained. This organism did not prove fatal in subsequent inoculations.

October 27 the eye was entirely closed. The agglutinated lids were separated and a wedge-shaped mass of necrotic exudate was easily removed. This covered the entire inner surface of the lower lid, being thicker at the exposed or free margin.

In section it was found to be composed very largely of broken-down cells. The cornea had an opalescent appearance and was decidedly flattened. A nonpathogenic bacillus was obtained in agar cultures from the eye.

November 9, the fowl appeared to be improved. The eye was covered with a yellowish exudate which emitted a peculiar, offensive odor. It was easily removed leaving a roughened, nearly healed surface. The cornea was opaque and depressed. The exudate from the mucosa of the mouth had sloughed, leaving a healed surface.

The fowl was chloroformed for further examination. It was much emaciated. The internal organs appeared to be normal. Tubes of bouillon inoculated from the liver, spleen, and blood remained clear.

II.

A review of the disease found in the 18 fowls shows that while there are many dissimilarities in the lesions in the different fowls they can not be easily construed to represent different diseases. Although much of the confusion which characterizes the popular conception of "roup" is apparent in the fragmentary history obtained of the outbreaks, a more careful study of the individual cases will show that there is a striking similarity between them. The lesions in the fowls examined were for the greater part localized on the mucosa of the organs in the head. Although all of the fowls which died were much emaciated, there were, with few exceptions, no lesions or evidence of organic disease in the internal organs. The cause of death and the extreme emaciation is difficult to explain in those cases where the lesions were confined to one eye or to the mucosa of the nares, excepting on the supposition that some poisonous or toxic substance was absorbed from the seat of the disease. In those cases where the lesions were in both eyes, or in the mouth and throat, difficulty in finding or swallowing food affords a rational explanation.

A study of the lesions revealed the interesting fact that in some cases the exudate was of a croupous character, and in others of a diphtheritic nature. In a considerable number of the fowls the exudate was more or less sloughed from the underlying tissue. The hypothesis is suggested by these varied conditions that perhaps the apparent croupous exudate is the first stage of the diphtheritic condition, and that the sloughed mass of decomposing exudate so frequently found is the last or advanced stage. There is a certain amount of evidence to support such an hypothesis, both in the description of fowl diphtheria in Europe and in the cases here recorded. The three stages or varieties of lesions which represent the types of this disease as encountered in these investigations may be more definitely defined as follows:

(1) An exudate of a serous or muco-purulent character in the conjunctiva or nasal cavities. Ordinarily this condition can not be recognized in the mouth. The mucosa in these cases is apparently but slightly altered. (Fowls Nos. 3, 4, 5, Outbreak I, and No. 71, Outbreak III.)

(2) The mucosa over a small or larger area is covered with a spreading exudate of a grayish or yellow color. It is firmly attached to the

mucous membrane, and when removed leaves a raw, bleeding surface. Sections through this exudate and the subjacent tissues show that the epithelial layer is destroyed, and the underlying tissue infiltrated with cells. The extent of the infiltration varies in different individuals. (Fowl 59, Outbreak I.)

(3) The mucosa is covered with a thick mass of exudate, varying in color from a milky white to a lemon yellow or brown. It is easily removed, leaving a more or less granular and healed surface. This sloughed mass is frequently dried at its margins to the adjacent tissue. It emits a strong putrid odor, due to decomposition. The drying of the margins prevents the fowl from expelling the exudate after it becomes separated from the underlying tissue. (Fowl 63, Outbreak V.)

The evidence to support the supposition that the three forms or types of exudate described are different stages in the same morbid process, as gathered from the post-mortem notes and bacteriological study of the cases here reported, may be summarized as follows:

(a) Abnormal conditions, representing the intermediate and connecting links between the specific types of lesions, were frequently encountered.

(b) Although but one form of exudate was usually present in a single fowl, there were marked exceptions, in which two, and in one case the three, forms were coincident. Thus in fowl No. 75 (Outbreak III) the eye was covered with a sloughed exudate. In the posterior nares there was a layer of muco-purulent substance, and on the mucosa of the mouth were areas of a diphtheritic exudate. Fowls Nos. 69 and 73 furnish examples where the diphtheritic process and the sloughed exudates were present.

(c) The same species of a pathogenic bacillus was associated with each form of exudate. It was almost invariably found with the first and second, but rarely with the third.

In the fowls which died, the exudates were for the greater part in the advanced stage, although there were several fatal cases in which the lesions were restricted to an abnormal quantity of a serous or muco-purulent, more or less viscid, exudate in the conjunctiva or nasal cavities. The best illustration of the diphtheritic process was found in certain of the fowls killed for examination.

The distribution of the lesions shows that the conjunctiva was most frequently affected. The exudate in the nasal cavities was in a few cases undoubtedly the result of the coagulation of the liquid which had passed during the course of the first stage from the conjunctiva through the lachrymal duct into the nares. In certain of the other cases, however, the lesions appeared in the nares only (fowls Nos. 3, 4, and 5). Inadvertently, in these cases no material for sectioning was saved from the mucosa of the nares or conjunctiva. Sections of the exudate, with subjacent tissue from the cornea and the mouth, show that there is a cell

infiltration into the mucosa which destroys the epithelial layer and frequently the submucous tissues to a considerable depth. (See Pl. VI.)

The fact should not be overlooked, as pointed out in the post-mortem notes, that the disease in the eye was confined to the conjunctiva and the cornea, the posterior portion remaining apparently normal. Although the lesions were found more frequently in the eye, the number of fowls examined was not large enough to admit of the conclusion that this is generally the case.

From the observations thus far made the provisional theory is entertained that the three forms of the exudate—serous or muco-purulent, diphtheritic, and sloughed mass—represent three stages in the course of the same disease. It is easily understood that fowls examined in the first stage would be said to be affected with a catarrhal condition of the mucosa of the eyes or nares. It is highly probable that in many cases the disease never reaches the second stage (see fowls Nos. 3, 4, 5, and 71), and if these cases alone were examined the diphtheritic condition would not be suspected. It appears, however, that in the majority of cases the disease runs its course, and membranes are formed, slough, and recovery follows. It is further presumable that the disease in question appears sometimes in a virulent and destructive form. I am in possession of statements from poultry raisers which show that there are occasionally epizootics of a disease characterized by exudates in the eyes, nose, or mouth, which runs a rapidly fatal course. It appears that it is such outbreaks which have been reported in Europe as diphtheria, and not the low form of chronic disease which I have encountered.

It is highly probable that there are many affections of fowls resembling somewhat closely the lesions found in the cases examined which are etiologically different. There is much doubt respecting the cause of the apparently sporadic cases of this disease. Whether they differ other than in the degree of virulence of the specific organism from the rapidly fatal disease sometimes reported can not be determined until such epizootics can be thoroughly studied. It is hoped that the investigation of this disease or class of diseases may be continued until the doubtful points concerning their nature and cause are clearly explained, and efficient methods of prevention and treatment are determined.

BACTERIA ASSOCIATED WITH THE LESIONS.

It has already been shown that bacteria were not found in the internal organs or blood of the affected fowls when they were examined before post-mortem changes occurred. It has also been stated that a non-motile, pathogenic bacillus was found associated with the lesions, especially in the first and second stages, in a considerable number of the fowls.

The examination of the exudates for bacteria was attended with many difficulties, owing to the presence of a large number of sapro-

phytes.¹ The method found to be the most trustworthy for the isolation of the pathogenic bacteria was the subcutaneous inoculation of experimental animals with the exudates. If the inoculated animals died, pure cultures of the pathogenic organism were obtained from the blood, liver, or spleen. If they survived, they were subsequently chloroformed and cultures of the parasitic bacteria obtained from the tissue at the point of inoculation.² By this process the saprophytes were destroyed in the tissues and only those organisms encountered which were suspected to be of more or less etiological importance.

The success of the animal inoculation over the culture methods in isolating the nonmotile pathogenic bacillus is explained from the fact that it is not vigorous in its growth on culture media and that it was ordinarily crowded out by more vigorously growing saprophytes. It is further presumable that in the older exudates its virulence is gradually lost (see fowls Nos. 1 and 59), so that its presence could not be detected readily by animal inoculation. If rabbits had been inoculated, however, with large quantities of the exudate from the more advanced disease a few additional cases containing the pathogenic—but attenuated—bacillus would undoubtedly have been found. The severe local lesions produced in rabbits by the injection of such material caused them, with few exceptions, to be abandoned. It is evident, in view of the results obtained, that rabbits were the most efficient experimental animals to use in this work, and that fowls were the most refractory. The scarcity of rabbits, however, led to the use of other animals in a few cases.

Cover-glass preparations made from the lesions of the affected fowls were carefully stained and examined.³ Those prepared from the surface of the exudate and from the necrotic masses generally contained innumerable bacteria, but no predominating species was observed. Similar preparations from the base of the exudate in the more recent stages contained a large number of short bacilli with the ends rounded. These forms frequently exhibited a polar stain. Several preparations were

¹A large number of species of bacteria were isolated and studied somewhat carefully. Among them are several quite interesting forms, some of which were suspected to be of more or less economic importance. Prominent among these was a bacillus which resembled morphologically and in certain of its cultural characters the bacillus of tuberculosis. It was not pathogenic for guinea-pigs or fowls, and did not take the Koch stain.

²It is of interest to note that several varieties of colon bacteria were isolated in this way. One of these possessed such variations from the colon bacillus in its cultural manifestations and it was possessed of such marked pathogenesis that for a time it was suspected of bearing some causal relation to the disease. It was briefly described in a footnote on page 43.

³In both fresh and stained preparations a long spirillum was found to be quite abundant. It was from 2 to 4 μ in length. Thus far I have been unable to detect it in artificial cultivations.

stained for tubercle bacilli, but with negative results. A summary of the results obtained from animal inoculations is appended in tabulated form

Animals inoculated with the exudate from affected fowls.

Animals inoculated.	Inoculated with exudate from—	Date of inoculation.	Results.	Remarks.
Rabbit No. 117...	Eye, fowl No. 1.....	Dec. 28, 1893	Died Feb. 10, 1894.	Rabbit very much emaciated; no bacteria found in its organs.
White mouse.....	do	Jan. 23, 1894	Died Jan. 27, 1894.	Enlarged and discolored spleen; a pathogenic motile bacillus.
Do.....	do	do	Died Jan. 29, 1894.	Spleen enlarged; nonmotile pathogenic bacillus.
Rabbit No. 144...	Nares, fowl No. 3...	Feb. 4, 1894	Died Feb. 28, 1894.	Septicæmia; nonmotile pathogenic bacillus.
Rabbit No. 145...	do	Feb. 24, 1894	Died Feb. 25, 1894.	Do.
Rabbit No. 97...	Nares, fowl No. 4.....	do	do	Do.
Rabbit No. 149...	Nares, fowl No. 5.....	Feb. 29, 1894	Died Mar. 1, 1894.	Do.
Guinea-pig 222...	Mouth, fowl No. 61...	Oct. 9, 1894	Chloroformed Oct. 13, 1894.	Local lesion contained <i>Bacillus coli communis</i> .
Guinea-pig 223...	Nares, fowl No. 62.....	do	Chloroformed Oct. 15, 1894.	Do.
Guinea-pig 209...	Mouth, fowl No. 59...	Oct. 20, 1894	Chloroformed Oct. 25, 1894.	Local lesion contained nonmotile pathogenic bacillus.
Fowl No. 50.....	Eye, fowl No. 65.....	Oct. 24, 1894	Remained well....	No local reaction.
Fowl No. 54.....	Eye, fowl No. 68.....	Oct. 27, 1894	do	Do.
Fowl No. 55.....	do	do	do	Do.
Rabbit No. 215...	Eye, fowl No. 69...	Nov. 2, 1894	Found dead Nov. 5, 1894.	Pleuritis; hemorrhages in the intestines; nonmotile pathogenic bacillus.
Rabbit No. 223...	Eyes, fowl No. 71...	Nov. 5, 1894	Found dead Dec. 19, 1894.	Pleuritis and peritonitis; nonmotile pathogenic bacillus.
Rabbit No. 226...	Nares, fowl No. 75...	Nov. 22, 1894	Found dead Nov. 23, 1894.	Septicæmia; nonmotile pathogenic bacillus.

The table shows that the pathogenic bacillus was obtained from the lesions of fowls Nos. 1, 3, 4, 5, 59, 69, 71, and 75, or nearly 50 per cent of those examined. It is a significant fact that the bacillus was obtained from all the fowls but one (No. 61) where the lesions either in the eye, nares, or mouth were not far advanced. In one case (No. 73) no inoculations were made. The almost constant appearance of this bacillus in the more recent lesions renders the apparent small percentage of cases from which it was isolated of much greater importance than it would at first be considered. A comparative study of the bacteria from the different fowls showed that those obtained from fowls Nos. 1, 59, 69, and 71 were attenuated in varying degrees, while those from the other cases were sufficiently virulent to destroy rabbits within twenty-four hours. This variation in the degree of virulence of the cultures obtained from different fowls is interesting and important. There was no difference in their cultural characters. A glance at the post-mortem notes will show that in the fowls from which the attenuated forms were obtained the lesions were apparently older or more advanced than in the other cases.

In previous investigations¹ rabbits were inoculated with the nasal and pharyngeal secretions of several healthy fowls. These rabbits

¹ Bulletin No. 3, Bureau of Animal Industry, 1893, p. 47.

remained perfectly well. Although septic bacteria are known to be common in the secretions covering the normal mucosa of the upper air passages of several species of domesticated animals, such organisms have not been reported from the mucosa of healthy fowls.

In 1890 two fowls¹ which died very suddenly at the experiment station of this Bureau were examined. One of them exhibited a croupous exudate extending from the larynx into the trachea, and the other a swollen condition of the mucosa of the mouth and œsophagus. No pathogenic bacteria were obtained from the fowl suffering from the exudate, but from the other were obtained pure cultures of a bacillus not distinguishable from that of swine plague.

It is impossible to positively identify the pathogenic bacillus found associated with the lesions in this disease with the one described by European writers as the cause of fowl diphtheria. Morphologically it is similar to the one described by Loeffler, but its pathogenesis is different. This may be due, however, to difference in the degree of virulence. There is much obscurity in the reference to the properties of the bacilli found by other investigators as the probable cause of diphtheritic affections of fowls and birds, as their descriptions are exceedingly meager, being often limited to the morphology and possibly the character of the growth on one or two of the more commonly used culture media.

In those species of pathogenic bacteria which have been more fully described a more accurate comparison is possible. Thus, the bacilli of fowl cholera, swine plague, and rabbit septicæmia are found to be comparable with the bacillus about to be described, and found in the lesions of this disease of fowls. While it is not my purpose to discuss at this time the identity or relationship of these bacteria, it is important to know that a bacillus associated with the peculiar lesions of this disease should be so similar to the one described by European writers as the cause of fowl cholera. The more essential properties of the bacillus found, apparently the etiological factor, in the lesions of the diphtheritic disease of poultry which I have studied are appended:

DESCRIPTION OF THE NONMOTILE PATHOGENIC BACILLUS.

Morphology.—A nonmotile, rod-shaped organism 0.8 to 1.5 μ long and from 0.8 to 1.2 μ thick. The ends are oval, and the shorter forms appear to be nearly spherical. In bouillon they are frequently in short chains and in clumps. When stained with the aniline dyes in cover-glass preparations made directly from animal tissues they exhibit a light center, occasionally showing deeply stained poles. In preparations from cultures this character is much less marked. No capsule has been positively demonstrated, although certain preparations suggest its existence. It stains readily with the aniline dyes ordinarily used. It does not retain the coloring matter when treated after the Gram method.

Culture characters.—On agar at 36° C. the growth is not vigorous. It is of a neutral gray color, with a glistening, moist-appearing surface. The growth is slightly viscid, and adheres quite firmly to the agar surface. The condensation water becomes

¹ Special Report on Swine Plague, Bureau of Animal Industry, Department of Agriculture, 1891, p. 158.

faintly clouded. Within agar the colonies appear as minute grayish dots. The growth is completed in about forty-eight hours. In agar plates (double disks) it emits a disagreeable, pungent odor, similar to that observed in cultures of the swine-plague bacillus.

It does not grow in alkaline, peptone gelatin. It does not develop on potato.

Milk remains unchanged in appearance for six weeks. Cultures which had grown for several days were boiled. No change was produced.

Alkaline bouillon at 36° C. becomes uniformly clouded in twenty-four hours. The reaction becomes acid in from one to two days. It does not grow in acid bouillon.

In bouillon containing sugar the growth was slightly more vigorous. In the fermentation tubes the liquid in both branches becomes cloudy. Gas is not produced. In bouillon containing dextrose and saccharose the alkaline reaction is changed to a strongly acid one in twenty-four hours; the bouillon containing lactose remained alkaline.

In a weak solution of peptone containing salt it grew feebly, but gave a decided indol reaction. The phenol reaction was not detected.

Thermal death point.—This organism is destroyed in bouillon cultures heated in a water-bath for five minutes at 58° C. An exposure of ten minutes to a temperature of 54° C. was not fatal.

Effect of drying.—The bacillus is destroyed in twenty-four hours by drying on cover-glasses at the temperature of the laboratory.

Effect of low temperature.—Dry cover-glass preparations were exposed in a sterile test tube to a temperature varying from zero to a few degrees above for a period of seventeen hours. At the end of that time they were placed in tubes of bouillon and put in the incubator. These tubes contained pure cultures of this bacillus on the following day.

Disinfectants.—A few preliminary tests showed that this organism was very sensitive to ordinary disinfectants. To determine more accurately the strength of the solution and the time necessary for a fatal exposure, Dr. C. F. Dawson kindly carried out an extensive series of experiments, the more important results of which are appended:

Commercial sulphuric acid in one-eighth of 1 per cent solution destroys in thirty minutes; in one-fourth of 1 per cent it kills in ten minutes.

Lime water destroys the organism in one minute. When diluted four times (0.015 per cent lime) it requires thirty minutes. Carbolic acid in strengths of 0.5, 1, and 2 per cent destroys this bacillus in sixty, five, and one minutes, respectively.

The frequency with which sulphur fumes are used to disinfect chicken houses led to the testing of their efficiency on this organism. The methods and results reported by Dr. Dawson are as follows:

"A drop of bouillon culture was placed upon a sterile cover-glass inside a large glass jar which had been specially prepared for the test. About a half teaspoonful of sulphur was placed upon a sheet of asbestos and ignited. The lid of the jar was put on and in a short time the jar was filled with the white fumes. In three hours the cover-glasses were carefully removed and dropped into a tube of sterile bouillon and placed in an incubator. They invariably remained clear."

Pathogenesis.—Rabbits inoculated subcutaneously with 0.1 cc. of the virulent bouillon cultures died in from eighteen to thirty-six hours with lesions similar to those produced by swine-plague bacteria of a virulent type. The organs contained large numbers of oval bacteria exhibiting a polar arrangement of the protoplasm. Those inoculated in a similar manner with the attenuated bacilli lived several days. They exhibited more or less severe local lesions and exudates on one or more of the serous membranes. Adult fowls inoculated subcutaneously with 0.5 cc. of a fresh bouillon culture of the virulent form remained well. A young fowl (about 6 weeks old) inoculated in a similar manner with 0.3 cc. died on the fourth day. The kidneys were abnormally yellow in color and contained large quantities of urates.

Other organs were apparently normal. No bacteria were found in cover-glass preparations from the organs, but tubes inoculated with the blood and liver developed into pure cultures of the bacillus injected.

Several white and gray mice were inoculated subcutaneously with pure cultures. One of the white mice died. The others remained well.

Several efforts were made to produce the local disease by injecting pure cultures of this bacillus into the nasal cavity of fowls. These gave invariably negative results. Cultures were also fed in large quantities to several fowls without producing the disease.

The fact that diphtheritic lesions were not produced in fowls with this bacillus appears to be an argument against its specific relationship to the disease. Loeffler produced the disease in pigeons with the bacillus he isolated. In these investigations the conditions under which the inoculation experiments were made were presumably quite different from those surrounding the fowls which developed the disease. The variations in the degree of virulence of the organism from the different fowls renders it difficult to determine whether the infection depends on the virulence of the bacillus, or a predisposing condition on the part of the fowls. The etiological relation of this bacillus to the disease in question, however, is neither demonstrated nor affirmed, although the evidence in hand is quite enough to warrant the working hypothesis that it is, to a certain extent at least, an exciting agent in the production of the accompanying lesion.

PREVENTION AND TREATMENT.

The nature of this disease, as determined by the foregoing investigations and the published statements of a large number of poultry raisers, indicates that it can be prevented, and if introduced it can be cured. Although it will be necessary to determine experimentally the efficiency of curative agents on a considerable number of fowls before formulas for treatment can be laid down, several important suggestions present themselves at this time, especially in reference to preventive measures.

In order to prevent this disease it is evident that many conditions must be strictly complied with. The character of the food and the general sanitary conditions, including cleanliness, ventilation, and the temperature of the poultry houses, must be considered. Undoubtedly there is much to be learned in connection with the proper care of poultry, especially in rural districts where it is probable that many methods now in use will, after a careful investigation, be found defective. The determination of these details must necessarily be made by those actually engaged in the raising of poultry. In addition to the general sanitary methods, the following rules should be observed:

(1) Fowls which have an exudate on any of the mucous membranes of the head, or which have come from flocks in which such a disease exists or has recently existed, should not be introduced among other poultry.

(2) If the disease appears in one or more fowls of a flock they should be immediately separated from the well ones. If possible, the source of the infection should be determined and removed.

(3) The quite common practice of allowing fowls from different flocks to run together during the day should be discouraged.

(4) Care should be taken to avoid the possibility of bringing the virus of the disease from affected flocks in the dirt or excrement which naturally adheres to the shoes in walking through an infected chicken yard. The same care is necessary in the interchange of working implements, such as shovels, hoes, etc.

It is evident to any careful observer that the fact is too often overlooked that fowls, owing to their method of living, are more liable to infection than other farm animals. This is especially true when they are allowed to run at random, as they too frequently are, picking their living from the garbage pile and barnyards, or securing even more unwholesome food. There is little doubt that many so-called outbreaks of contagious disease among fowls are simply enzootics brought about by improper care. The efficiency of these few suggestions in reference to the prevention of this disease is demonstrated by the success of certain poultry raisers who adhere strictly to the teachings of sanitary methods.

The wide distribution, the large number of fowls affected, and the usual chronic course of this disease render it one of the few poultry affections for which curative measures promise to be of practical value. Although prevention is the safest of cures, when the disease is once introduced as it is in a very large number of flocks, the necessity for remedial treatment is apparent, and where economy is to be considered should be recommended. The practice sometimes followed of destroying all of the affected birds should be discouraged. Although experiments have not been made to test the efficiency of remedies already recommended, or to investigate the practicability of others, the testimony of many practical poultry raisers is, as previously stated, to the effect that the disease is amenable to treatment.

The most certain of the known methods of treatment is the local application of certain disinfectants, among which a weak solution of carbolic acid appears to be the most satisfactory. The fact that the lesions are so much exposed renders the disease especially favorable for topical applications. The administration of mild stimulants has also been recommended. In addition to the medicinal treatment, it is of much importance that the affected fowls be provided with proper food and kept in dry, warm, and well-ventilated apartments. In the course of this study a considerable number of badly affected fowls fully recovered in the animal room of this laboratory with no treatment other than a warm, dry atmosphere.

If the disease has reached its third stage, it is frequently necessary to remove the sloughed exudate, which is retained on account of the

attachment of its margins, before the application of the disinfectant. A few fowls have been received where it was evident that death would soon have followed from starvation or a closure of the respiratory passages, and which were immediately relieved and cured by removing the obstructing exudate. There are certain disinfectants and stimulants recommended, which I am advised will give good results, if administered early in the course of the disease, by mixing them with the food and thus eliminating the necessity for individual application:

THE RELATION OF DIPHTHERIA IN FOWLS TO PUBLIC HEALTH.

A comparison of the bacillus of diphtheria in man (Klebs-Loeffler) with the one described by Loeffler as the cause of diphtheria in fowls, shows that morphologically and in their pathogenesis for experimental animals the two organisms are in no way alike. There is also a marked difference in the nature of the exudates in fowls and in man. The non-identity of these diseases has been clearly pointed out by Ménard.¹ Although these diseases are shown by several observations to be unlike in their etiology and the character of their lesions, the transmission of fowl diphtheria to the human species, and vice versa, is affirmed by several writers.

Gerhardt² reports 4 cases of diphtheria among 6 workmen who had charge of several thousand fowls, many of which died of diphtheria, in Wesselhausen, Baden. There were no other cases of diphtheria in the neighborhood, and the evidence was quite conclusive that the disease was contracted from the affected fowls. It is also stated that an island on the northeastern coast of Greece had been free from diphtheria for at least a third of a century, when a dozen turkeys, several of which were diseased, were introduced. Soon afterwards diphtheria appeared in a house near the garden where the turkeys were kept. The disease became epidemic on the island causing the death of 36 people, or over 40 per cent of those attacked.

Debrie³ reports briefly the clinical history of 6 cases of diphtheria which occurred in the garrison of Sebdou, and states that while the sixth case (2 were fatal) was still under treatment in the hospital 10 fowls kept in a house not far from the hospital were attacked with diphtheria, and exhibited symptoms strikingly like those present in the human beings. Five of the 10 fowls died, and two heads were sent to Arloing, who confirmed the diagnosis of fowl diphtheria. The fowls were fed by a hospital attendant, and it was ascertained that an identical outbreak had occurred among the fowls at a neighboring place from which one of the 6 cases of human diphtheria had been brought. Debrie is inclined to the view that human diphtheria is transmissible to fowls and fowl diphtheria to man.

¹ Revue d' Hygiène, tome XII (1890), p. 410.

² Revue f. Thierheilkunde u. Viehzucht, Bd. VI (1883), p. 180.

³ Reviewed in Centralblatt f. Bakteriologie, Bd. XIII (1893), p. 730.

Cole¹ reports an interesting case near Jacksonville, Ill. A flock of fowls became affected with a disease characterized by an exudate on the mucosa of the head. Some of the exudates emitted a foul odor.² As the weather was cold, one of the chickens was taken into the house where a child about 2½ years old fondled it. Four days later the child was taken sick apparently with diphtheria, from which it died. There were no other cases in the neighborhood, and the affected chicken was the only possible source of infection.

The diphtheritic disease of fowls reported by Loir and Ducloux (*loc. cit.*) in Tunis, in 1894, spread to the people of that place, resulting in an epidemic of serious proportions. Ménard refers to the fact that men employed to feed young squabs contracted diphtheria by blowing the masticated food into the mouth and crop of squabs suffering with that disease. Schrevens³ reports several cases of diphtheria in children in which he traces the source of infection to certain poultry.

Although the number of reported cases of the transmission of fowl diphtheria to the human species, and vice versa, is small in comparison with the extent of the disease in poultry, the evidence that such a transmission is possible is quite sufficient to discourage the careless handling of diseased fowls. It is a quite common practice, especially in the rural districts, to bring the sick chickens into the house for treatment, where the children of the household are allowed to fondle them at will. It is not improbable that when this disease is thoroughly investigated the number of cases of direct infection from this source will be found to be much larger than it is at present supposed. Until such investigations are satisfactorily completed the indiscriminate handling of diphtheritic chickens, especially by children, and the exposure of fowls to the infection of diphtheria in the human species, whereby they may become carriers of the virus, should be strenuously avoided.

CONCLUSIONS.

The preceding investigations, though preliminary, are important in bringing together certain existing facts and in a measure correlating certain confusing and contradictory theories in reference to a few of the more common so-called diseases of poultry. Although many important questions, especially concerning the etiology, are as yet unsatisfactorily answered, the facts elicited are of much value as a basis for future investigation. The definite results which have been obtained, and which mark the progress made in our knowledge of fowl pathology, may be summarized in the following determinations:

- (1) Many of the so-called diseases of fowls which are characterized

¹Archives of Pediatrics, XI (1894), p. 381.

²Cole's description of the disease shows that it was undoubtedly the same as the one I have studied.

³Bulletin de l'Acad. Royale de Méd. de Belgique, VIII (1894), p. 380.

by an affection of the mucous membranes of the head, and popularly designated as "roup," diphtheria, influenza, and sometimes cholera, resemble each other so closely in their manifestations that they may be considered as belonging to one and the same disease. This disease is distinct from the rapidly fatal malady which is better known as fowl cholera.

(2) The lesions, as usually encountered, are diphtheritic in nature. In the advanced stages the accumulating exudates decompose and emit a putrid odor. In the earlier stages they are frequently of a serous or muco-purulent character, and not infrequently fowls die before the diphtheritic condition appears.

(3) The same species of a pathogenic bacillus is associated with apparently different forms of lesions, more particularly in the serous or muco-purulent and diphtheritic. This bacillus is not distinguishable from the one described by European writers as the cause of fowl cholera and can not be differentiated in parallel cultures from the bacillus of swine plague. It is comparable in certain respects with the supposed specific organisms of certain diphtheritic diseases of poultry and birds. Its causal relation to this disease, however, is not demonstrated.

(4) This disease usually runs a slow, chronic course from which the majority of the affected fowls recover. Its long duration enables the lesions to be more or less modified from the effect of external contamination. Outbreaks in which the disease runs a rapidly fatal course are reported, but thus far they have not been encountered in the investigations of this Bureau.

(5) This disease, or, as may subsequently be found, diseases, is amenable to treatment. The treatment consists largely in good hygiene, and the removal of the accumulated exudate when the disease has reached the advanced stage. The local application of disinfectants is indicated.

(6) A large number of slight or more severe enzootics among fowls, due to local unsanitary conditions, are popularly considered as outbreaks of some contagious disease.

(7) Judging from the results of these investigations and the recorded experience of certain poultry raisers, it is highly probable that this malady would have been prevented in a large proportion of the flocks in which it now exists if careful sanitary methods had been followed and precautions taken against the introduction of the disease through the purchase of affected fowls.

A STUDY OF A BACILLUS OBTAINED FROM THREE OUTBREAKS OF FOWL CHOLERA.

By VERANUS A. MOORE.

Although a large number of rapidly fatal outbreaks among fowls are reported annually from the central and southern sections of the United States, very little is positively known of the nature and cause of the disease producing them. In 1879-80 Dr. D. E. Salmon¹ investigated certain epizootics among fowls in North Carolina, in which he found a disease comparable to the one described as *cholera des poules* (fowl cholera) in France. With the exception of his investigations, which were made before the development of bacteriological methods now considered essential and which were restricted to an inquiry into the nature of the disease in very few outbreaks, we have no definite knowledge of the disease known as fowl cholera in America. In Europe this disease has been extensively studied and the specific organism carefully described by several observers; the descriptions of the specific bacillus differ somewhat in reference to certain properties, but, without attempting a review of the voluminous literature on the subject, suffice it to say that it is generally accepted that the bacillus of fowl cholera is very closely related to, if not identical with, the bacillus of rabbit septicæmia. The results recorded by Dr. Salmon show that he obtained a similar organism from the fowls he examined, but unfortunately the condition of bacteriological science at that time did not permit the determination of certain important differential biological properties.

The further inquiry into the nature and cause of this disease of fowls has been attended with much delay on account of the difficulty in procuring suitable material. Although this may appear paradoxical in the presence of so many reports of epizootics, a moment's reflection upon the existing condition will reveal its truthfulness. It was shown in the previous article that there is a great looseness in the use of the term "fowl cholera," so that many, if not all, of the forms of local, infectious, and contagious diseases of poultry are frequently designated by it. Again, there is a hesitancy among poultry raisers to report the existence of a disease in their flocks, especially before its seriousness is

¹ Reports of the Department of Agriculture, 1880, 1882.

demonstrated by the death of several individuals when it is usually too late for an investigation. Repeatedly, reported epizootics have been visited by different investigators of this Bureau, but invariably the conditions found precluded the determination of the nature of the disease which had already run its course and disappeared.

In the summer of 1894 three serious outbreaks of fowl cholera were reported, and from each of them a single fowl was received for examination. From the organs of each of these fowls a nonmotile pathogenic bacillus was obtained in pure cultures. As I was unable to study the outbreaks in the field, the investigations here recorded are necessarily confined to the study of the organism obtained from these fowls and the disease produced by feeding and inoculation experiments. Fowls and rabbits inoculated with pure cultures of the bacillus, isolated from each of the affected fowls, produced similar fatal results, showing that very little if any difference existed in the degree of virulence possessed by the different cultures. A comparative study of their cultural characters and physiological properties showed them to be identical. The study of this organism, however, showed that it possessed certain properties which have not heretofore been attributed to the bacillus of fowl cholera, and which are not possessed by the bacillus of swine plague or rabbit septicæmia, bacteria generally supposed to be identical with it. The history of the outbreaks, so far as learned, and the conditions found in the fowls examined are appended:

I.

April 7, 1894, a dead fowl was received from Mr. S., who resides near the city of Washington, D. C. He had lost nearly all of his fowls (about 50) with cholera during the preceding few weeks, the one brought to us being the last to die. He was requested to notify us if the disease continued in his flock, and to send all the fowls that were subsequently attacked. As no other fowls were received, it is presumable that the disease had disappeared and that the fowl examined was the last to be affected.

The fowl received was a hen about 2 years old. She was in a good condition. The liver, spleen, kidneys, and lungs, appeared to be normal. The crop contained a considerable quantity of dirt, kitchen scraps, corn, and other food. In the abdominal cavity were two large blood clots and a broken egg. The mucosa of the head normal. Comb pale.

Cover-glass preparations made from the liver showed no bacteria. Tubes of agar and bouillon were inoculated from the liver and blood. On the following day these contained pure cultures of a nonmotile bacillus. It was fatal to fowls, rabbits, guinea-pigs, and mice.

II.

August 8, 1894, a dead fowl was received by express from Mr. S., of Tackett's Mills, Va. A postal card from him bearing date of August 8, addressed to the Department of Agriculture, reads in part as follows: "I send you by Adams Express a hen with genuine cholera. I have lost about 80 hens and pullets in the last month." * * * About three weeks later a letter was received from Mr. S. in which he stated that he had lost about 100 hens and pullets in the last two months from fowl cholera.

The autopsy of the fowl received showed that death had occurred sometime before its arrival. The organs were somewhat discolored from post-mortem changes. The liver was fatty, kidneys yellowish in color, due to an excess of urates in the tubules. Heart muscle pale. The contents of the intestines were greenish and semiliquid in consistency. Mucosa of cloaca had a yellowish color. Tubes of bouillon and agar were inoculated from the liver. The following day the tubes contained pure cultures of a nonmotile bacillus, which upon subsequent study was found to be identical with the bacillus obtained from the fowl examined from the first outbreak.

III.

In August, 1894, while engaged in other investigations, Dr. Theobald Smith had the opportunity of examining a fowl which died in an outbreak of fowl cholera on Block Island, R. I. The fowl died August 13. It was kept on ice until August 15, when it was carefully examined. It came from a flock of about 70 fowls. The disease was reported to have begun in June, and at the time this fowl died only about 14 or 15 were still living. The autopsy notes made by Dr. Smith show that the organs appeared to be normal. Four cultures were made from the spleen, liver, and blood. On the second day following these contained a moderately vigorous growth of a nonmotile bacillus.

August 31: Dr. Smith gave me these cultures for further study. They were found to be identical and pure. A series of inoculations and a careful study of the morphology and physiological properties of this organism showed that it was identical with the bacillus obtained from the other two fowls.

Although the data concerning the nature of the disease in the three outbreaks is exceedingly meager, the facts in hand are quite enough to show that it was practically the same in its manifestations in the three localities. From other sources I have learned that fowl cholera, or a rapidly fatal disease known as such, is not uncommon in the vicinities where two of the outbreaks occurred. No one can doubt that, if the history given is correct, the disease in the flocks from which the fowls came was of a highly contagious, or at least infectious, nature. The presence of the same species of a pathogenic bacillus in the organs of the three fowls is evidence supporting such a conclusion. As the history of the disease in each flock and the examination of the fowls received showed the absence of diphtheritic lesions, it is necessary to consider the disease in question either fowl cholera, in the general acceptation of that term, or to add a new disease to fowl pathology, which, from our limited knowledge of this disease in the United States, does not appear to be justifiable.

DESCRIPTION OF THE BACILLUS.

Morphology.—A nonmotile, rod-shaped organism, varying somewhat in size according to the media in which it develops. In tissues of fowls or rabbits it is from 1.4 to 1.8 μ long and from 1 to 1.3 μ thick. No spores or vacuoles have been discovered. The ends are rounded, so that in the shorter forms it could easily be mistaken for a micrococcus. In the tissues it frequently appears in small clumps. Involution forms are common. In cultures on agar it is more slender than in tissues. When examined in a hanging drop preparation, especially at the edge, it frequently shows a marked polar arrangement of the cellular protoplasm. In these preparations there is observed a marked dancing motion of the organisms. In old bouillon cul-

tures short chains composed of bacilli united end to end are sometimes observed. It stains with the ordinary aniline dyes, but retains the coloring matter very feebly, or not at all, when treated after the Gram method.

Cultural characters.—Agar.—On this medium at 37° C. the growth is moderately vigorous. It has a grayish, glistening appearance. Isolated colonies are from 1 to 2 mm. in diameter, convex, with sharply defined borders. Agar plates emit a peculiar penetrating odor.

Gelatin.—In this medium the growth is less vigorous. In stick cultures it is more abundant along the line of inoculation than on the surface. Isolated colonies are about 0.25 mm. in diameter, appearing as homogeneous bodies to the unaided eye, but slightly granular under low magnification. They have a grayish-yellow color.

Potato.—On the surface of potatoes a delicate grayish-yellow growth appears after about forty-eight hours. Frequently there is no development, owing, presumably, to the acid reaction.

Alkaline bouillon.—In twenty-four hours alkaline bouillon becomes uniformly clouded. Reaction acid. The bouillon becomes clear after several days. The growth settles with the formation of a small quantity of friable sediment. The bacteria live for at least four weeks.

*Acid bouillon.*¹—Very faintly clouded; acid reaction. Slight sediment, which is not viscid.

Milk.—No change is produced in the appearance of milk for about four weeks. It then begins to change to a clear opalescent-appearing fluid. In about six weeks the milk has a decidedly opalescent appearance. Strongly alkaline in reaction. The addition of a few drops of acetic acid precipitates the casein. A microscopic examination shows that the fat globules are destroyed. This condition is presumably due to the alkali produced by the growth of the bacteria. This appearance of milk is not distinguishable from that produced by the bacillus of hog cholera.

Fermentation of sugars.—Alkaline bouillon containing 1 per cent dextrose in fermentation tubes becomes uniformly cloudy throughout. Strongly acid in reaction. Similar tubes containing saccharose and lactose become clouded throughout, but they remain alkaline in reaction, the degree of alkalinity increasing with age. Gas is not produced during the growth of the bacillus in bouillon containing sugars.

Indol and phenol.—Cultures in a peptone solution give a strong indol reaction when treated after Kitasato's method. No phenol reaction could be obtained when cultures were treated according to Weyl-Lewandowski.

Thermal death point.—Tubes of bouillon inoculated with several drops of a fresh bouillon culture, exposed in a water bath to a temperature of 58° C. for fifteen minutes or longer, did not develop. Those exposed for ten minutes or less developed into pure cultures of the inoculated bacillus. An exposure at 54° C. for thirty minutes produced no deleterious effect.

Effect of disinfectants.—A one per cent solution of carbolic acid was fatal in five minutes. A one-fourth of one per cent solution of commercial sulphuric acid destroyed life in ten minutes. Lime water was fatal in ten minutes, and a solution composed of one part lime water and three parts distilled water was equally as effective in one hour. Sulphur fumes were also fatal in the test applied by Dr. C. F. Dawson. A drop of a bouillon culture was placed upon a sterile cover-glass inside a large glass jar which had been specially prepared for the test. About a half teaspoonful of sulphur was placed upon a sheet of asbestos and ignited. The lid of the jar was put on and in a short time the jar was filled with white fumes. In three hours time the cover-glasses were carefully removed and dropped into tubes of sterile bouillon. These tubes were placed in an incubator. They invariably remained clear.

¹ Bouillon prepared from beef without neutralizing or rendering alkaline by a solution of soda or potash.

Effect of low temperature.—Bacteria dried on cover-glasses were placed in sterile test tubes and exposed in a temperature of from zero to a few degrees above for seventeen hours. This exposure did not appreciably retard their subsequent multiplication in bouillon.

The effect of drying.—This bacillus withstood drying at the ordinary temperature on cover-glasses for seven days.

THE NATURE OF THE INOCULATION DISEASE.

The disease produced in the experimental animals with pure cultures of this bacillus is, when compared with the described effect of the bacillus of fowl cholera, exceedingly interesting.

Fowls inoculated in the wing vein with 0.3 cc. of a fresh bouillon culture died in from three to eight days, usually on the fifth or sixth day. The temperature begins to rise on the second day after inoculation. It reaches 109° to 111° F.¹ the day before death occurs. In cases where the fowls live from five to six days they appear perfectly well for at least three days, when the feathers begin to have a slightly ruffled appearance. The exposed portion of the head usually, but not invariably, becomes pale. Toward the last they sit in a crouched position, with the head drawn close to the body. They refuse food, but usually take more or less water. There is sometimes a slight diarrhea. That portion of the excrement secreted by the kidneys becomes yellow.

The changes observed at post-mortem are not conspicuous. The liver is moderately enlarged, soft and fatty. The spleen is occasionally enlarged. The kidneys are marked with yellowish lines, due to the injection of the tubules with urates. The intestinal mucosa is sometimes hyperemic and sprinkled with punctiform hemorrhages, especially in the rectum. The contents of the intestines are, as a rule, greenish in color and normal in consistency. The mucosa of the cloaca is frequently of a yellowish color, due to urates. The heart muscle is pale. In a few cases, where the fowls resisted for an unusually long period, tubercle-like nodules were observed in the heart and lungs.

The most marked pathological changes were found to occur in the blood. Within twenty-four to forty-eight hours after an inoculation into the veins a diminution in the number of the red corpuscles and an increase in the number of leucocytes was determined. This decrease in the number of the red cells and the increase in the white ones continued until death occurred. This is illustrated by the notes from a somewhat typical case.

Fowl No. 82.

[Inoculated February 6.]

Date.	Temperature. F. ^o	Number of red cor- puscles.	Number of white cor- puscles.	Remarks.
February 6.....	107.4	3,744,444	22,222	
February 7.....	109	3,417,391	26,087	Apparently well.
February 8.....	108.2	2,784,700	55,000	Do.
February 9.....	108.4	2,807,692	76,925	Do.
February 11.....	107.4	3,481,818	90,909	Feathers ruffled; refuses food.
February 13.....	110.2	2,133,333	100,000	Very quiet; comb pale.
February 14.....	108	2,530,000	140,000	Fowl dies later in the day.

Unfortunately the changes produced in the blood of fowls by bacterial diseases are so little known that the extent to which this marked change in the blood is characteristic of this disease can not be determined until further investigations with other pathogenic bacteria are made.

¹The normal temperature of fowls, as determined by taking the temperature of the fowls used before inoculation, varies considerably, ranging from 104° to 108° F.

The method of destruction of the red corpuscles is not as yet satisfactorily explained. In his report on fowl cholera Dr. Salmon (*loc. cit.*) illustrated leucocytes surrounding the red corpuscles, but the marked diminution of the red cells was not determined, although he speaks of the pale color of the blood. In a few preparations of fresh blood I have observed similar phenomenon, and also have seen portions of red cells within the granular leucocytes. The determination of the extent of this mode of destruction of the red corpuscles, however, necessitates further investigation. It is of interest to call attention to certain other observations in connection with their destruction.

In carefully heated cover-glass preparations of healthy fowl's blood stained with eosin and methylene blue the nuclei are colored a deep blue, and the cellular protoplasm surrounding the nucleus is stained with the eosin. In similar preparations made from the blood of the affected fowls there are a greater or less number of corpuscles which do not take the eosin stain. In these the portion of the corpuscle surrounding the nucleus remains unstained or becomes slightly tinted with the blue. It occasionally contains one or more vacuoles, and the margin is frequently broken. In some instances a considerable portion of the corpuscle has disappeared. There are a few free nuclei.

Fowls fed with pure cultures and with the viscera of birds dying of this disease usually succumb in from eight to fifteen days. The subcutaneous inoculation with moderate quantities of pure cultures gave negative results.

The organs and the blood of fowls dying from the inoculation disease contained comparatively few bacteria. This was shown in the cover-glass preparations and also in cultures. Frequently the inclined surface of agar rubbed with a large piece of the liver or spleen, or a clot of blood, contained on the following day isolated colonies. In sections the bacteria were frequently found lying in clumps within the capillaries.

In rabbits the disease resembles very closely that produced by attenuated hog-cholera bacteria, although the fatal dose is much larger than for that organism. Subcutaneous inoculations with 0.1 to 0.2 cc. are rarely fatal. When death follows the lesions are characterized by slight local reactions, necrotic areas in the liver, enlarged and discolored spleen, and an infiltration of cells into the follicles of the vermiciform appendix and glands about the ileo-cecal valve. An intravenous inoculation of 0.2 to 0.3 cc. of a fresh bouillon culture produces death in from two to three days. In these cases the spleen is engorged with blood, the liver swollen, and the intestinal mucosa contains hemorrhagic areas. A larger dose produces death in from eighteen to twenty-four hours. An intravenous injection of a small quantity of culture produces lesions similar to those following the subcutaneous inoculation. Cover-glass preparations from the liver, spleen, and blood made soon after death show few elongated bacteria. If a rabbit is allowed to lie for twenty-four hours after death cover-glass preparations show innumerable oval bacteria, which usually exhibit a polar stain.

A guinea-pig inoculated in the abdominal cavity with 0.2 cc. of a bouillon culture died in seven days. The autopsy showed about 50 cc. of a clear, lemon-colored serum in the abdominal cavity. The viscera were covered with a grayish membranous exudate. The pleural cavity contained a large quantity of serum. Lungs collapsed. Subcutaneous inoculations of 0.5 cc. proved fatal in from six to eight days. There was a purulent infiltration into the subcutis about the point of inoculation. Spleen enlarged, dark colored, and friable. Liver pale and usually fatty. No intestinal lesions. Comparatively few bacteria in the organs as shown in cover-glass preparations.

White and gray mice inoculated subcutaneously with 1 to 2 drops of bouillon culture died in from three to five days. The only pronounced macroscopic lesion observed was an enlarged and discolored spleen. Mice inoculated similarly with a much smaller quantity (a single small loop of culture) remained well.

It was thought that perhaps the bacillus was attenuated because the fowls were among the last to die in their respective outbreaks. On that account several series of inoculations were made in different animals for the purpose of increasing its virulence, the culture from the fowl in the first outbreak being used. These gave negative results. Several other experiments are now in progress for the purpose of accelerating its pathogenesis. It seems, however, that its virulence is not easily changed by any of the ordinary methods. After nearly a year's preservation in subculture it is as rapidly fatal as when first isolated.

The bacillus here described differs from the description of the bacillus of fowl cholera (rabbit septicæmia or swine plague) in the following particulars:

- (1) Morphology.
- (2) Its effect upon milk.
- (3) Its effect upon sugars.
- (4) Its degree of virulence for rabbits and fowls.
- (5) The nature of the inoculation disease, especially in rabbits.

The morphological changes consist in a slight increase in size and the feebleness with which it takes the polar stain. The fact is interesting, however, that the polar stain is well marked when the bacillus is allowed to multiply in the dead animal body.

The saponification of milk is very marked. This property belongs to the hog-cholera group of bacteria, and it has not occurred in parallel cultures of the swine-plague bacillus or other members of that group, in which it is generally conceded by authorities in bacteriology the fowl-cholera bacillus belongs.

The bacilli of rabbit septicæmia and swine plague produce acids in bouillon containing dextrose and saccharose, while this bacillus changes dextrose only. I have not had an opportunity of studying the bacillus of the European disease, and consequently this variation is assumed on the authority of the statements that these species are identical.

The virulence of this bacillus is much less than that ordinarily recorded for fowl cholera. Dr. Salmon produced fatal results in fowls by the subcutaneous injection of minute quantities of a pure culture, while this organism requires for fatal results an intravenous injection of a much larger amount. In rabbits the dissimilarity is much greater. The bacillus of fowl cholera is said to destroy rabbits with acute septicaemia within twenty-four hours after a subcutaneous inoculation with small quantities of pure culture, but this organism produces a disease characterized by certain definite anatomical changes.

The theory that possibly the variation in the lesions is due to an attenuation of the bacillus or a resistance on the part of the animal does not hold, as the disease produced in rabbits with this organism is different from that obtained with attenuated rabbit-septicæmia bacteria. It has frequently been pointed out in the publications from this laboratory that attenuated rabbit-septicæmia or swine-plague bacteria pro-

duce local infiltrations and an inflammation followed by an exudate on one or more of the serous membranes.

Another point of difference, taking the bacillus of fowl cholera to be identical with that of swine plague, is the inability to render rabbits immune to the disease by previous injections of sterilized cultures. A carefully conducted experiment was made in which 10 cc. of sterilized bouillon culture was injected intravenously in repeated small doses in two rabbits. They were subsequently inoculated with a minimum fatal dose of pure culture. They died with the control rabbits. Such negative results are invariably obtained with hog-cholera bacteria, but many experiments with swine-plague bacteria show that such treatment will quite as surely produce a pronounced resistance to that disease.¹

There are, however, certain similarities in the disease produced in fowls with the one studied by Dr. Salmon in his investigations in North Carolina. These are, for the greater part, the rise in temperature, the period of incubation, yellowish color of the urates, paleness of the comb, and the refusal of food. The gross pathological changes are also comparable. The diarrhea was less marked in the disease I have produced.

The study of this organism and its pathogenesis are as yet too incomplete to admit of further comparisons at this time. The fact has been shown that in the inoculation disease there is slight or no diarrhea, and pronounced pathological changes occur in the blood. Should these conditions be found to exist in the disease as it occurs in epizootics it is evident that the term fowl cholera is an unfortunate one. It has further been pointed out that the bacillus obtained from three outbreaks of reported fowl cholera differs materially from the organism generally accepted, and oftentimes described by European investigators as the specific cause of that disease. It is quite possible that there are several varieties of fowl cholera, and that the bacillus here described is the etiological factor of one of these forms. The extent to which this bacillus is distributed in this country as the cause of epizooties among fowls can not be determined until further investigations are made.

¹ Bulletin No. 6 of the Bureau of Animal Industry, 1894, p. 65.

ON A PATHOGENIC BACILLUS OF THE HOG-CHOLERA GROUP ASSOCIATED WITH A FATAL DISEASE IN PIGEONS.

By VERANUS A. MOORE.

HISTORY OF THE DISEASE.

In July, 1894, an investigation was made into the nature of a reported epizootic among pigeons at Vineland, N. J. The disease was found, however, to be restricted to the cotes belonging to Mr. A. H. Hawley, and while it was said to be very destructive, its course was slow, and the heavy loss which it occasioned was due to its continued existence and spread. The only symptom observed was a general emaciation, or "going light," as expressed by pigeon fanciers. He had examined post-mortem a large number of the birds, but the only apparent pathological change noticed, other than the loss of flesh, was an exceedingly pale heart muscle.

In addition to the affection mentioned, a few pigeons had died from a disease known as "megrims," which is characterized by emaciation and a peculiar turning of the head from one side to the other, with occasional paroxysms of flying about, apparently in an aimless manner. The economic importance of this disease, however, was said to be insignificant, and it is considered here in connection with the more serious affection on account of the identity of the bacilli isolated from the diseased pigeons.

At the time of my visit Mr. Hawley had a pigeon in the advanced stage of each of these apparently different diseases, and several others which he said showed beginning symptoms. As these diseases appeared to have followed outbreaks of a rapidly fatal affection which had occurred at variable intervals among his pigeons during the preceding few years, a brief historical sketch of the disease in his cotes is of special interest.

Mr. Hawley stated that he began to raise pigeons early in 1889. At first he kept about 160 pairs. For nearly three years his pigeons remained well. Late in the fall of 1891 he purchased 50 pairs, aged about 10 months, from the Fanciers' Pigeon Club, of Philadelphia. About four weeks later these pigeons began to die from a disease des-

ignated by him as cholera. About one-half of the purchased pigeons died. The disease did not spread to the pigeons formerly kept on the place. A second and more destructive outbreak occurred in the spring of 1892. During the summer two other quite severe outbreaks occurred. In August, 1893, he lost 125 birds from the same cause. At this time the disease was unusually rapid in its course, the pigeons being sick for only twelve to fourteen hours. The cotes in which the disease occurred were thoroughly sprayed with kerosene emulsion and the floors were washed with carbolic acid. They were vacated and left unoccupied until the spring of 1894, when 75 pairs of pigeons, selected from the other cotes, were placed in three of the apartments, 25 in each, and 17 pairs were purchased and put in a fourth apartment of the same house. The four apartments were arranged side by side, occupying the entire building. The pigeons remained well for about a month, when cholera broke out in two (first and third) of the apartments. About 15 birds died, when the disease was checked by the use of medicines.

Soon after the first outbreak of so-called cholera, late in 1891, pigeons began to die of the disease under investigation. The number of deaths continued to increase until the profit of the business was consumed and the extermination of the pigeons threatened. A record of the mortality kept during the first few months of 1894 showed that in February 83, in March 36, and in April 66 deaths occurred. In May and June the loss was about the same. In July a much smaller proportional number had died up to the time of the investigation. The prevalence of the disease among young pigeons was noted by Mr. Hawley, although old birds were occasionally affected. All of the affected pigeons were examined.

Pigeon No. 1.—This pigeon was found dead on the morning of July 19. It was about 6 weeks old. There was evidence of a slight diarrhea. It was much emaciated. The mucosa of the eyes, nares, and mouth was normal. The liver had a peculiar greenish color, due, presumably, to post-mortem changes. The kidneys were of a pale yellowish color. Intestinal mucosa normal. The heart muscle was exceedingly pale, fatty. Tubes of agar were inoculated from the liver and blood. These contained on the day following pure cultures of a motile bacillus belonging to the hog-cholera group, as will be shown in its subsequent description.

Pigeon No. 2.—This was an old pigeon. It had been sick for several days. It turned its head continually from one side to the other and walked with difficulty. The left eye was closed and contained a small quantity of mucus. It was killed for examination. Pigeon much emaciated. No lesions were found in thoracic or abdominal organs. In the subarchnoid space over the cerebellum and the posterior lobes of the cerebrum there was a grayish-yellow, friable exudate one-half to 1 mm. in thickness and easily removed. Subjacent brain tissue reddened; otherwise normal in appearance. The dura appeared to be normal. The exudate was composed for the greater part of degenerating cells and bacteria. Tubes of agar were inoculated from the exudate over the brain and from the heart blood. The latter remained clear; the former developed into pure cultures of a bacillus not distinguishable in its biological character from the one obtained from the organs of pigeon No. 1.

All of the suspected pigeons were killed and examined, but in every case the organs appeared to be normal and the culture media inoculated remained clear.

DESCRIPTION OF THE BACILLUS.

Morphology.—An actively motile bacillus, varying in size from 1 to 1.6μ in length and about 1μ in width. The size varies appreciably in different media. No spores have been detected. The ends are rounded in cultures, but in tissue they appear to be more pointed. In preparations from tissues it usually appears in pairs, united end to end. Frequently, however, it is observed in clumps. The flagella are easily demonstrated by Loeffler's process. The number of filaments vary, eight being the maximum number observed in a single bacillus. In preparations from tissue it usually exhibits a light center with a uniformly stained periphery. In preparations from cultures it usually shows very little, if any, unstained area. It does not take the Gram stain.

Cultural characters.—*Agar.*—On the inclined surface of this medium after twenty-four hours at 37° C. a grayish, glistening, nonviscid growth appears. When isolated the colonies are convex, 0.5 to 1.5 mm. in diameter. Edges sharply defined. The growth is increased slightly during the next twenty-four hours. In stick cultures there is a grayish growth along the course of the needle track.

Gelatin.—In this medium the growth is not vigorous. Surface colonies appear as small grayish dots. When magnified they are finely granular in appearance and of a yellowish tint. In older colonies there is a distinct band which is less granular in appearance about the circumference. It is not liquified.

Potato.—It forms on potato a thin, glistening growth, having a faintly yellowish color. When the reaction of the potato is very acid no growth appears.

Alkaline bouillon.—This medium becomes uniformly clouded after twenty-four hours. The reaction is faintly acid, owing to the fermentation of the muscle sugar. After a longer growth a thin, grayish membrane forms over the surface of the liquid and a grayish band frequently appears on the sides of the tube at the surface of the liquid. The reaction becomes strongly alkaline in old cultures. In *acid bouillon* the growth is feeble and the reaction is not appreciably changed.

Milk.—When this medium is faintly acid in the beginning it slowly becomes alkaline. In about three weeks it gradually changes to a clear, opalescent fluid, due, presumably, to the alkali¹ produced by the growth of the bacteria, as the milk at this time is very strongly alkaline. The addition of a few drops of acetic acid precipitates the casein. If the culture is allowed to remain in the thermostat for several weeks the volume of the milk shrinks, due to evaporation, and the remaining part becomes viscid and of a light brownish color.

Alkaline bouillon containing dextrose.—In this medium it produces a marked fermentation of the sugar with the formation of gas. The acid resulting from the fermentation gives the fluid a strong acid reaction. The gas produced (when collected) in the fermentation tube replaced 54.8 per cent of the liquid in the closed branch. The gas contained 35.7 per cent CO_2 (gas absorbed by potassium hydrate) and 64.3 per cent H (explosive gas).

In similar bouillon containing lactose and saccharose the liquid became uniformly clouded throughout both branches of the tube. No gas was formed. The reaction remained alkaline, and after some days the degree of alkalinity, as detected by litmus paper, was much increased.

A marked indol reaction was determined by Kitasato's method.²

Thermal death point.—This organism is destroyed in cultures by an exposure of ten

¹ It was shown in Bulletin No. 3, 1893, p. 43, that the addition of a few drops of a strong solution of caustic potash or soda would produce this appearance in milk.

² This consists in cultivating the bacillus at 37° C. in a sterilized solution composed of 1 per cent peptone and 0.5 per cent sodium chloride in distilled water for from one to three days. To these cultures 1 cc. of a 0.02 per cent solution of potassium nitrate, freshly prepared, and about 3 drops of chemically pure sulphuric acid are added; a violet-pink color indicates the presence of indol.

minutes to a temperature of 58° C. in a water bath. It resisted drying on cover-glass preparations made from bouillon cultures at the temperature of the room for eight days. Similar tests were not made with preparations made directly from the organs of animals.

Disinfectants.—The effect of lime and carbolic acid upon this organism shows that a solution of lime weaker than lime water is not effective, and that a 2 per cent solution of carbolic acid requires one hour to destroy life.

Pathogenesis.—*Rabbits* inoculated in the ear vein with 0.3 cc. of bouillon culture died in thirty-six to forty-eight hours. There was a general congestion of the internal organs. The intestinal mucosa was especially reddened in certain areas. A subcutaneous injection of a similar quantity of culture produced death in from four to six days. In these cases there was considerable purulent infiltration into the subcutaneous tissue about the place of inoculation. The liver was sprinkled with necrotic areas and the spleen much enlarged, dark colored, and friable. When a smaller quantity of culture was injected the local lesion was much more severe, and the follicles of the veriform appendix and also certain of those of Peyer's patches were infiltrated with cells, giving them the appearance of round, whitish dots. *White mice* died in from three to six days after a subcutaneous inoculation with one to three drops of bouillon culture. The only lesion observable was an enlarged and discolored spleen. *Guinea-pigs* died in from five to ten days after subcutaneous inoculations with from 0.1 to 0.2 cc. of a fresh bouillon culture. *Pigeons* resisted a subcutaneous inoculation with a small dose, but a larger quantity (0.2 to 0.5 cc.) injected into the circulation or pectoral muscle produced death in from two to eight days.

Two pigs inoculated with 5 cc. each of a bouillon culture, one subcutaneously and one in the femoral vein, remained apparently well.

The description of this bacillus shows that it undoubtedly belongs to the hog-cholera group of bacteria, although it differs in certain important properties from the specific bacillus ordinarily encountered in destructive outbreaks of hog cholera. As several varieties¹ of the hog-cholera bacillus have already been described, it is important to note the specific variations of the bacillus in question from the virulent form described² originally and the one which is generally meant when this organism is mentioned. The differences are as follows:

- (1) It is appreciably larger.
- (2) In bouillon a delicate membrane appears on the surface of the liquid and in old cultures a deposit is formed on the sides of the tube.
- (3) There is a marked indol reaction.
- (4) It is less rapidly fatal in small doses for experimental animals, and the lesions produced in rabbits are comparable to those following the inoculation with the more attenuated varieties. The negative results from the pig inoculations are parallel with many similar ones heretofore made with the virulent variety.

¹In Bulletin No. 6 of the Bureau of Animal Industry, 1894, Dr. Theobald Smith has pointed out the difference that exists in the morphology, cultural characters, and pathogenesis of the hog-cholera bacillus as encountered in different outbreaks of that disease. He also found that several bacilli associated with diseases other than hog cholera belong to the same group. These are a bacillus found by himself in a mare after abortion, *Bacillus enteriditis*, and *Bacillus typhi murium*.

²Second Annual Report of the Bureau of Animal Industry (1885), p. 212. It was called at that time swine-plague bacterium.

(5) It is slightly more resistant to the action of certain disinfectants.

The variations, with one exception, have already been noted in some one or other of the varieties described by Dr. Smith. It is of interest to note that of the seven varieties of hog-cholera bacillus which he has pointed out the indol reaction was markedly present in but one variety (*Bacillus cholerae suis* γ), and that a nonvirulent form. This is of interest, especially as the indol reaction is prominent in the colon group of bacteria. The greater resistance to disinfectants is also interesting.

The discovery of this bacillus in a disease of pigeons is of much interest in the accumulation of data concerning the distribution of this organism and the range of its disease-producing power. The source of infection is somewhat obscure, but the history points very clearly to its introduction with the pigeons purchased in Philadelphia. Accepting that as the source of infection, it is presumable that the acute disease breaking out at variable intervals was due to this organism, which for undetermined reasons became increased in virulence, or on account of certain conditions the pigeons were rendered more susceptible to it. A careful inquiry failed to reveal the existence of outbreaks of hog cholera in Vineland for many years. It was also learned that the disease found among Mr. Hawley's pigeons had not appeared in other cotes in that vicinity.

The conditions under which the affected pigeons were kept and the care exercised in their management seemed to be all that could be desired. The cotes, however, were located in a slight depression, the land surrounding them being appreciably higher, so that drainage is imperfect. The soil is quite sandy, however, and dries quickly after rains.

The fact was elicited in conversation with a number of pigeon raisers in southern New Jersey that a rapidly fatal disease, comparable to the one described by Mr. Hawley, does occasionally occur among pigeons where the source of infection can not be explained. I was unable to find such an outbreak, although one gentleman told me of recently losing 1,500 birds in one of these outbreaks. The results of the investigation at Vineland render a careful study of the so-called pigeon cholera of much interest. It is not improbable that future inquiry will find that these outbreaks are due to bacteria belonging to the hog-cholera group.

The appearance of this bacillus in the brain of the pigeon affected with the so-called "megrims" is of special interest. It was stated by Mr. Hawley that the old birds were affected in this way. This disease is reported by pigeon raisers to be invariably fatal and more or less common, although comparatively few birds are affected. In this case, however, it appears that the disease was etiologically the same as that which caused the large number of deaths among the younger pigeons, the lesions being localized on the brain. This fact renders the further

investigation of "megrims" important in order to determine whether the single case here reported is comparable in its etiology to the disease generally. The symptoms appear to be characteristic, so that the disease is readily recognized by pigeon fanciers.

The fact that pigeons, which are ordinarily quite refractory to hog-cholera bacteria, should be affected with a disease due apparently to an organism belonging to that group of bacteria is of much importance. Although it has been shown that several varieties of the bacillus described originally from outbreaks of hog cholera are associated with other diseases and in different species of animals, they appear not to have heretofore been encountered as the apparent cause of any disease in pigeons or other birds, with the possible exception of the bacillus described by Loeffler as the cause of pigeon diphtheria.

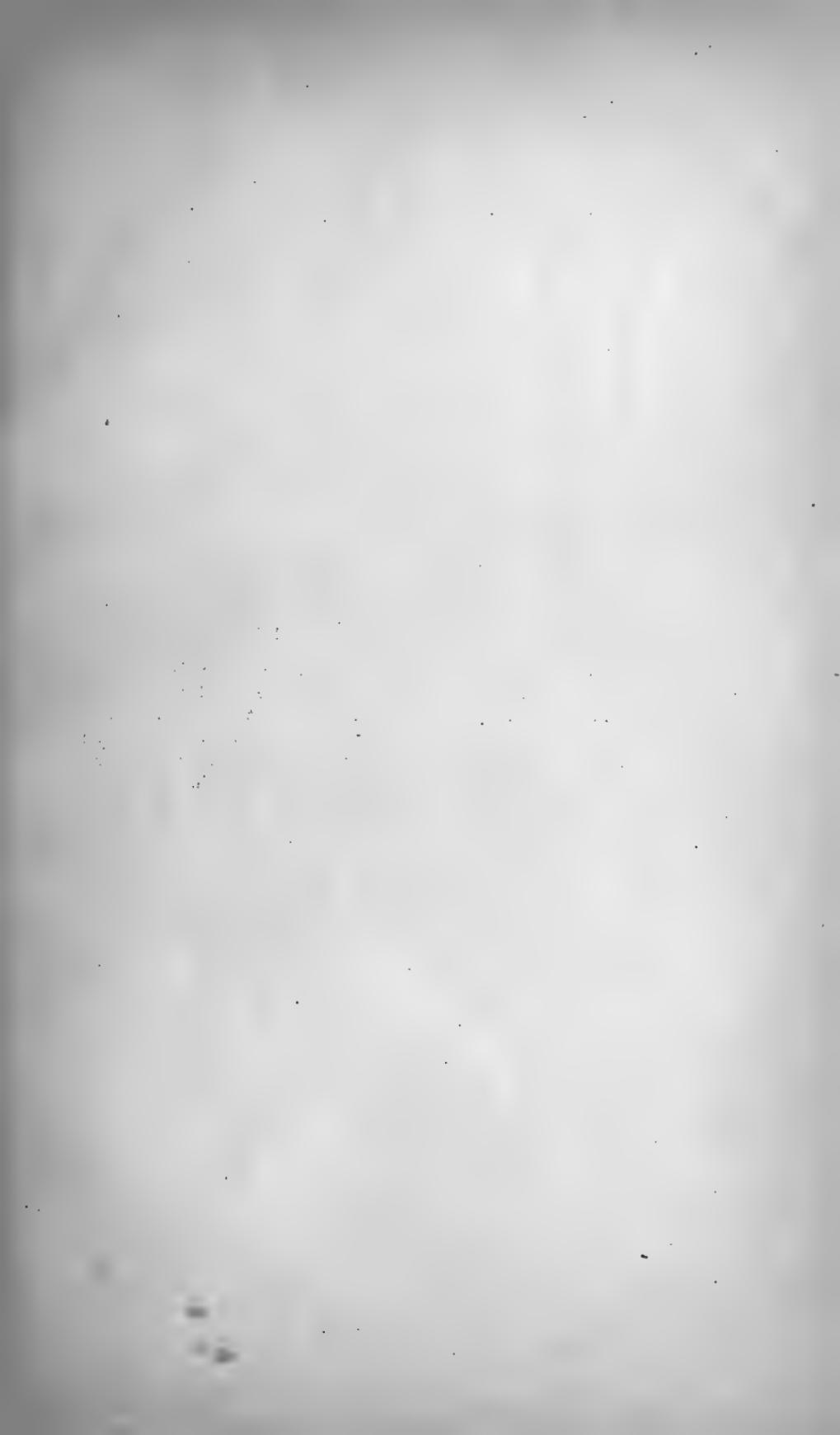
The importance of the results of this investigation, which was materially restricted by the limited number of examinations possible, is centered in a few determinations in reference to the hog-cholera bacillus.

(1) The discovery of a member of the hog-cholera group of bacteria apparently as the etiological factor in a chronic disease of pigeons.

(2) The appearance in pure culture of a variety of the hog-cholera bacillus from the exudate covering a portion of the brain of a pigeon killed in the last stages of the disease popularly known as "megrims."

(3) The presence of a marked indol reaction in cultures of a decidedly pathogenic bacillus of the hog-cholera group.

(4) The discovery of a variety of the hog-cholera bacillus in which the cultural characters and the physiological property of producing indol tend to assimilate to a marked degree those of closely related saprophytic forms, but which in its pathogenesis for experimental animals falls but little below that of the virulent form originally described. This striking commingling of saprophytic tendencies and highly parasitic powers tends to strengthen the proposed theory that the hog-cholera bacillus is derived from the closely related colon group of bacteria.



DESCRIPTION OF PLATE I.

- FIG. 1. Cæca of turkey No. 5 (somewhat reduced). Both are distended and filled with exudate. They are unusually short. (See fig. 2 on Pl. II.)
- FIG. 2. Liver of turkey No. 26 (dorsal aspect). Reduced to seven-ninths of original. The circular spots which are seen on the surface are shown in color in fig. 1 on Pl. III. The other surfaces of this liver presented a similar appearance. The liver is considerably larger than in health.

FIG. II



FIG. I







DESCRIPTION OF PLATE II.

- FIG. 1. Cæca of a healthy turkey, probably 9 months old. The central tube is the small intestine, the food passing downward in the direction of the arrow. Below, at the junction of the cæca with the intestine, the food is drawn into the cæca by suction.
- FIG. 2. One cæcum of turkey No. 5 (see fig. 1, Pl. I), cut open longitudinally. The middle portion of the tube is greatly distended and occluded with an exudate which is firm in consistency. The upper portion contains small stones which have passed down from the gizzard. The irregular thickening of the wall of the tube is shown by a faint line bordering the exudate.
- FIG. 3. The other cæcum of No. 5, cut transversely. The great thickening of the wall is shown, together with the presence of a loose plug within the tube, consisting of a firm exudate. Both this and the preceding figure were drawn from the alcoholic preparation. (Natural size.)
- FIG. 4. One cæcum of turkey No. 23, slit open and the mucous membrane exposed. The wall of this organ is very much thickened, as may be easily seen by comparing with the healthy tube of a turkey fully six months older (in fig. 1). On the mucous membrane are masses of exudate firmly attached to certain spots of increased thickness. (From an alcoholic preparation.)
- FIG. 5. Spots on the liver of turkey No. 34 (natural size). Drawn from an alcoholic preparation.

FIG. IV

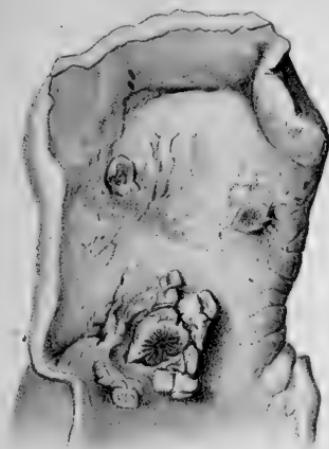


FIG. I



FIG. V

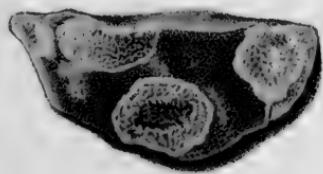


FIG. II



FIG. III



17897—No. 8—6

DESCRIPTION OF PLATE III.

FIGS. 1 and 2. Spots on the liver of turkey No. 5. (See photographic reproduction, fig. 2 of Pl. I.) The spots are shown both isolated and confluent. The reticulated structure of these spots is better brought out in the photograph referred to.

FIG. 3. Left lobe of liver of turkey No. 52 (convex surface). The large yellow area in the upper portion of the figure represents a mass of dead tissue penetrating nearly through the entire thickness of the liver. On the right the pale grayish spot represents diseased liver tissue which is undergoing repair. Similarly, the spots in the lower portion of the figure correspond to diseased regions partly healed. Several other spots readily detected in the specimen could not be clearly brought out in the figure. The diffuse change, probably reparative, is shown along the lower margin.

FIG. II

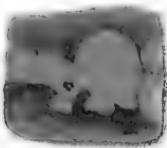


FIG. I

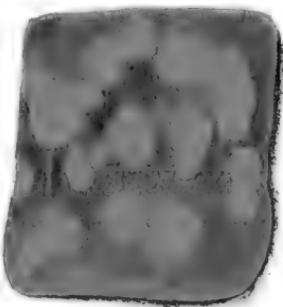


FIG. III







DESCRIPTION OF PLATE IV.

FIG. 1 ($\times 500$). A portion of the mucous membrane of a cæcum of turkey No. 26, cut transversely. The tissue was hardened in corrosive sublimate and alcohol, cut dry after embedding in paraffin, and stained in Delafield's hematoxylin and in eosin.

- (a) Nuclei of the epithelium of a crypt or tubule struck nearly tangentially by the section.
- (a') Mucin cell represented by a cavity in the section.
- (b) Spindle-shaped cells belonging to the reticulum of the tissue between the tubules.
- (c) Protozoa, single and in compact groups, situated within the meshes of the reticulum.
- (d) Multinucleated (giant) cell which has enveloped some of the protozoa.

FIG. 2 ($\times 500$). From the same section.

- (a) A large group of parasites inclosed in a space of the mucous membrane.
- (a') Single parasite.
- (b) A tissue cell in process of division.

FIG. 3. Section of the liver of turkey No. 34. Hardened in alcohol, cut dry in paraffin, and stained in hematoxylin and in eosin. A large collection of protozoa (a) occupying the space formerly occupied by the liver cells. They are probably somewhat forced apart by artificial pressure, for in other sections of the same liver compact masses of protozoa of equal size were not infrequently seen. The parasites are surrounded by capillaries (b) dilated and filled with red corpuscles; (c) round cells or leucocytes.

FIG. 4 ($\times 500$). From the same section as fig. 3. Protozoa (a) embedded in a granular matrix containing a few nuclei. Some of them largely disintegrated (b). (c) A capillary filled with red corpuscles. The matrix resembles the protoplasm of the liver cells.

FIG. I

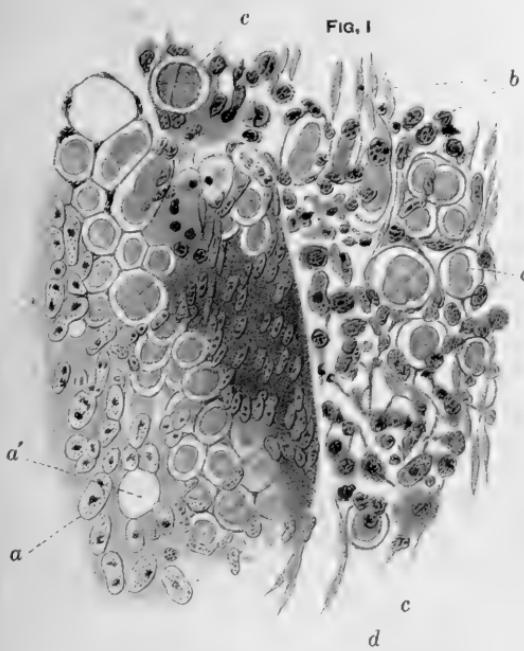


FIG. II

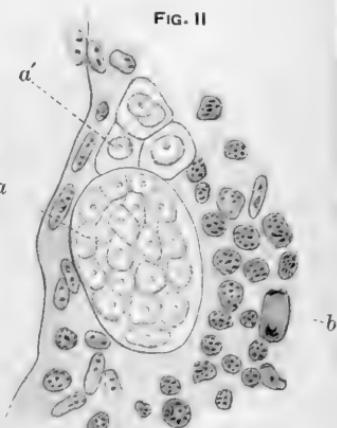
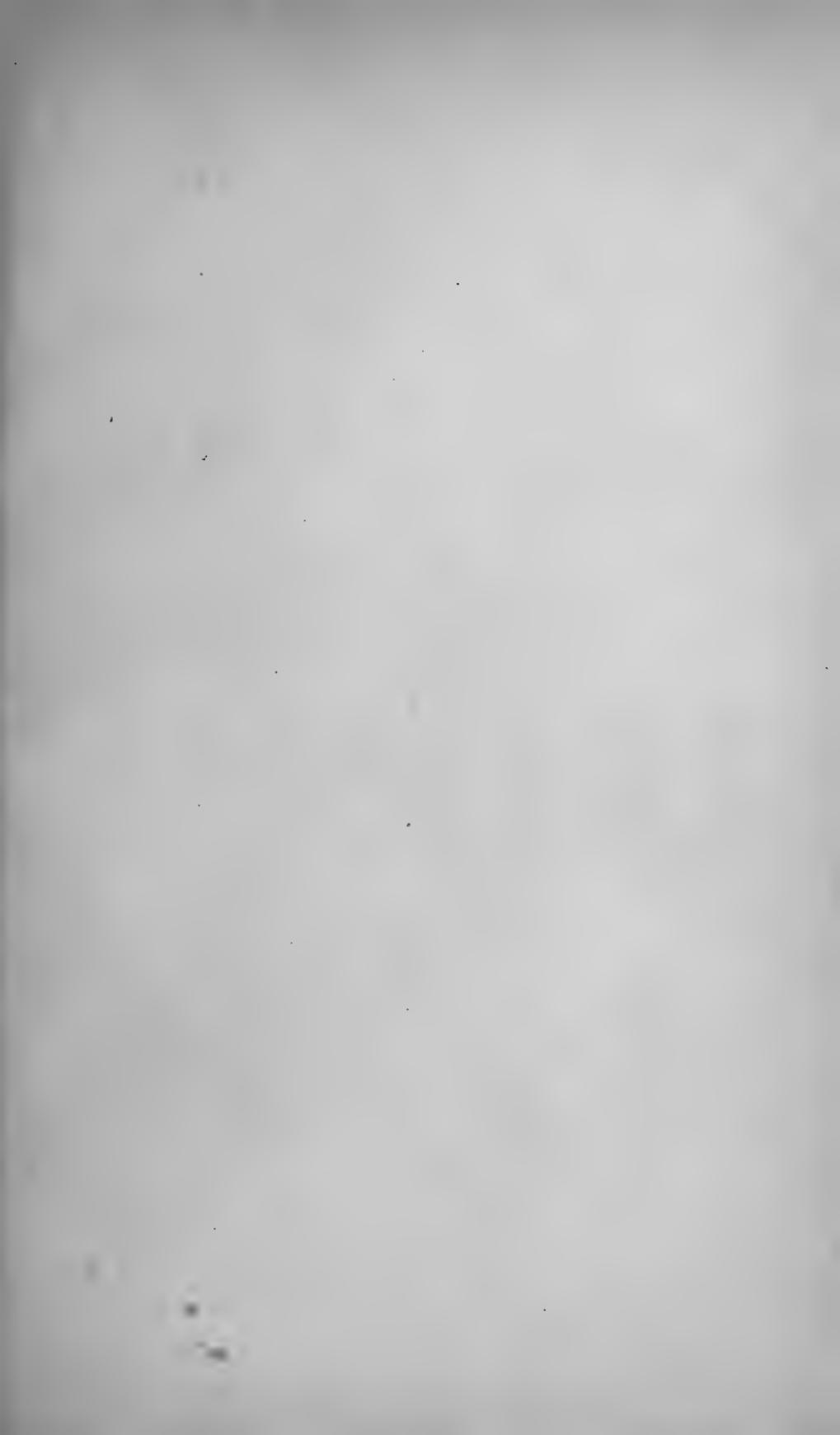


FIG. III



FIG. IV





DESCRIPTION OF PLATE V.

- FIG. 1. The protozoan parasites as they appear in crushed and teased fresh tissue ($\times 1,000$).
- FIG. 2. A parasite with granular body to which a fragment of a giant cell still adheres. The bodies in this fragment are fat globules ($\times 1,000$).
- FIG. 3. Section through the liver of No. 9. Hardened in alcohol and stained in hematoxylin and eosin ($\times 260$). A group of indistinctly demarcated giant cells containing a considerable number of vacuoles which indicate the position of inclosed microparasites. This group of giant cells is situated within what appears to be a thrombosed blood vessel. The coats of the vessel are faintly traceable. The surrounding tissue is involved in coagulation necrosis, presents a homogeneous appearance, and contains scattering nuclei.
- FIG. 4 ($\times 500$). A giant cell over 80μ in diameter among a group of ten or more slightly smaller ones found in the wall of cæcum of turkey No. 1. The mucous membrane had ulcerated away, and the greatly thickened remainder (3 to 4 mm. thick) was completely infiltrated with round cells. (a) Protozoa, two of them within the cell.
- FIG. 5 ($\times 500$). Transverse section through a crypt or tubule of the diseased cæcum of turkey No. 26. The lumen of the tubule is packed with minute bodies, probably flagellates. (a) Vacuole-like spots representing mucin cells. (b) Mitosis.
- FIG. 6 ($\times 500$). Blood from healthy turkey No. 12, dried as a thin film on a cover-glass and stained, after being fixed at 120° C., in Ehrlich's hematoxylin. (a) Red corpuscles, (b) leucocytes, (c) flagellates, very much broadened in appearance by the drying process.
- FIG. 7 ($\times 500$). Flagellate as seen in the fresh blood of healthy turkey No. 33.

FIG. III



FIG. I

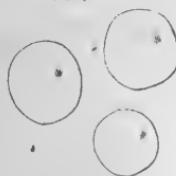


FIG. II

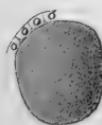


FIG. IV

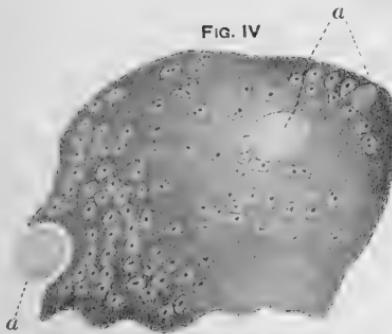
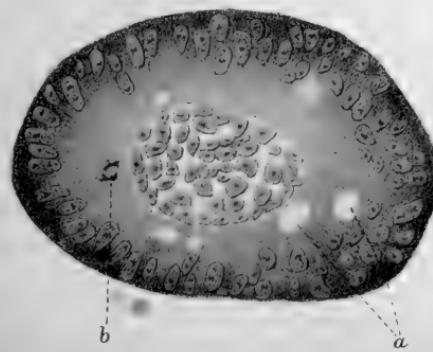


FIG. VII



FIG. V



a FIG. VI



b

c



DESCRIPTION OF PLATE VI.

- FIG. 1. The roof of the mouth of fowl No. 73, showing the position of the exudate. (Natural size.)
- FIG. 2. The floor of the mouth, showing the exudate over the tongue. (Natural size.)
- FIG. 3. Section of exudate with subjacent tissues, from mouth of fowl No. 59, showing destruction of the epithelium and cell infiltration of the underlying tissue ($\times 17$).
- FIG. 4. Sections of the cornea of fowl No. 65 after the removal of the mass of sloughed exudate ($\times 17$).

FIG. I



FIG. II



FIG. III

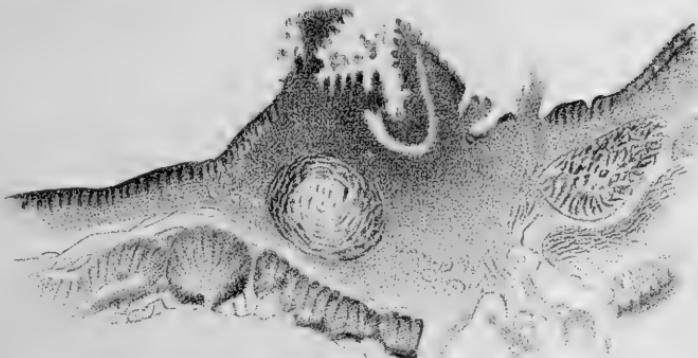
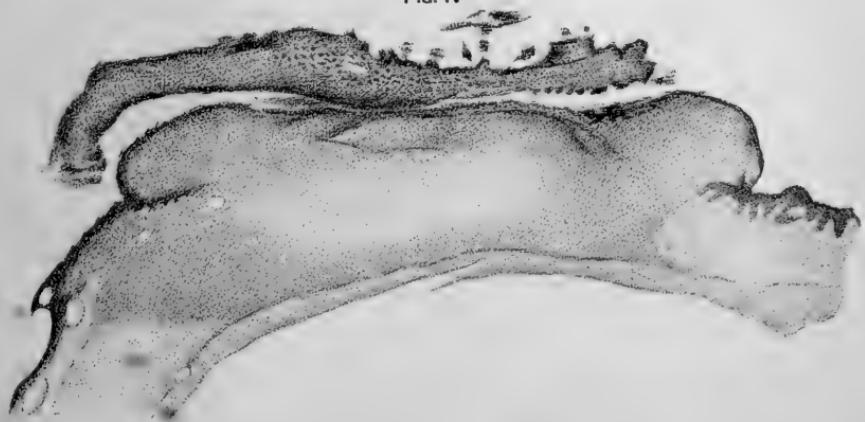


FIG. IV



INDEX.

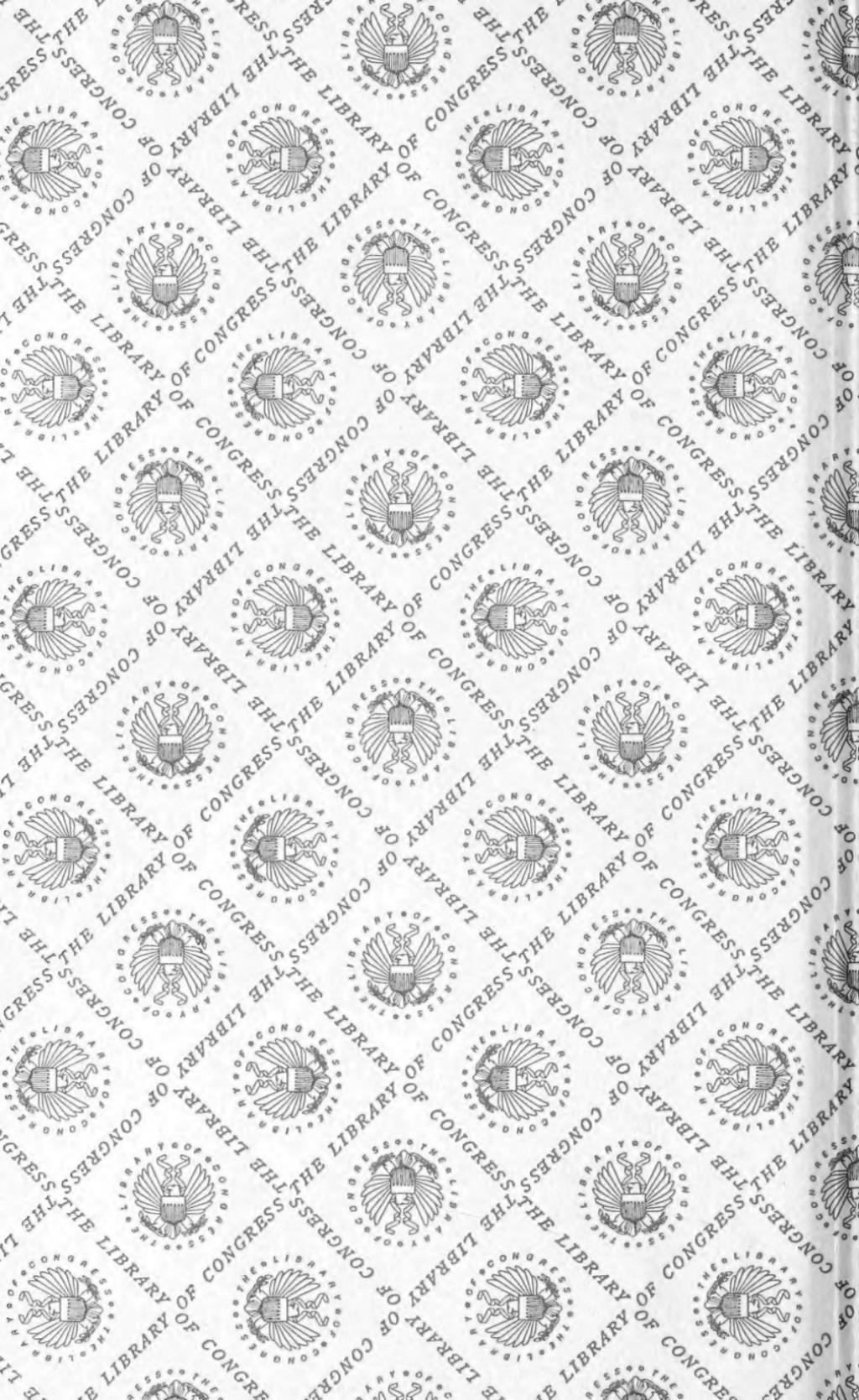
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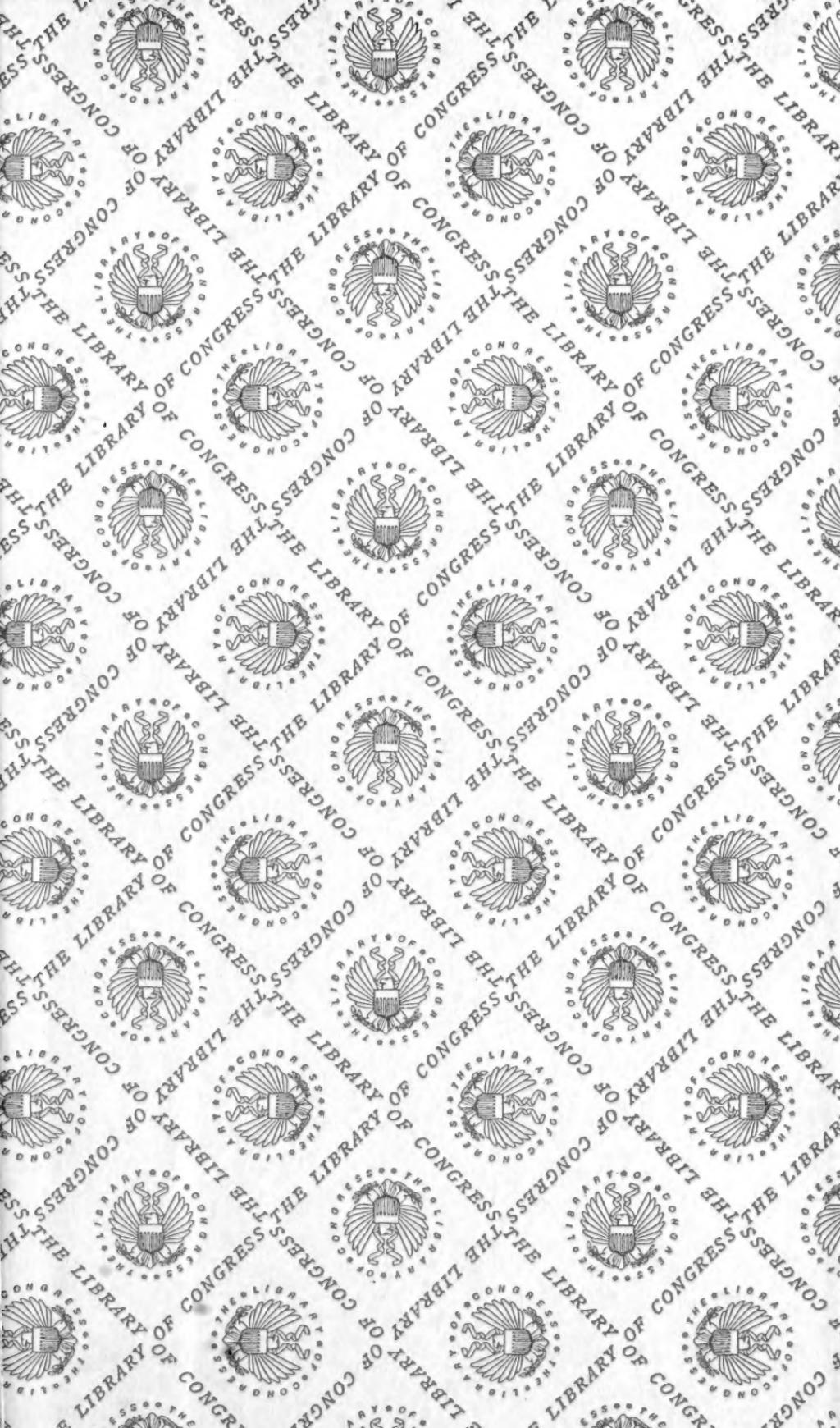
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